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FOCUS

Conservation of minor fruit genetic resources at the Botanical Garden, Bangladesh Agricultural University

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ABSTRACT

Minor fruits (MFs), a good source of micronutrients, can contribute significantly to the nutritional requirement of the rural population and be an alternative source to combat hidden hunger caused by micronutrient deficiencies mainly vitamins and minerals. A detailed survey was conducted to update the checklist of the MF collections at Botanical Garden under the Department of Crop Botany, Bangladesh Agricultural University (BAUBG) and their conservation priority in national/international perspectives. A total of 108 MF species, belong to 67 genera and 38 families, were collected and conserved at the BAUBG. Moraceae and Myrtaceae were the most dominant families with 8 taxa each followed by Rutaceae with 7 taxa, Arecaceae, Phyllanthaceae and Sapotaceae with 6 taxa each. Among the genera, Syzygium is the largest genus having 7 species followed by Citrus, Ficus and Garcinia with 5 species in each. Trees make up the largest proportion of MFs followed by shrubs, herbs and climbers. Although MFs are major sources of micronutrients, 97 taxa were also used in the treatment of various diseases in ethnobotanical literature. This study also contributes to the conservation database of plant genetic resources of BAUBG and the country as well.

***Keywords*:** Botanical garden, conservation, medicinal uses, underutilized fruits

INTRODUCTION

Minor fruits (MFs) are a group of fruits presently growing in a scattered and unattended way on roadsides, homestead land, wasteland, etc. (Roya and Bauri, 2019). These fruits are cultivated to a limited extent only, and with consumption and trade being more limited both geographically and quantitatively, although many are of considerable economic importance in their respective regional markets. In general, these are suitable for human consumption but relatively less palatable than other major fruits (Srivastava *et al*., 2017); MF species act as life support species in extreme environmental conditions and threatened habitats and have the tolerance to survive under harsh climatic conditions. The MFs are sometimes the only source of protective food to meet the vitamins and minerals requirements, and integral part of traditional foods of the people living in (remote) villages and tribal areas (Krishna *et al*., 2019). These fruits play a great role in improving the food and nutritional status of the local people due to their year-round availability (Pasha and Uddin, 2019). The MFs, synonymously called underutilized fruits, remain underutilized because of underestimation of their potential uses, little known outside its growing area, non-availability of their complete botanical information, inadequate research on their commercial exploitation, lack of knowledge on their food and nutritional value, promotion, popularization of very few fruit crops, fast disappearance of the ecosystem, and habitat destruction (Dandin and Kumar, 2016). Nowadays, for achieving the Sustainable Development Goals (SDGs), especially Goal #2, which calls for the eradication of hunger and all forms of malnutrition; MFs have become the focus of attraction. Because they are found harbouring nutritionally rich compounds, climate-resilient, resistant to biotic & abiotic stresses, rich in medicinal & nutritive value, source of breeding materials for crop improvement (Ghosh, 2017; Krishna *et al*., 2019). The MFs are rich in phytochemicals and micronutrients such as antioxidants, polyphenols, flavonoids, minerals and vitamins, which are essential for good health and nutrition, advancing physical and intellectual development. Many of the MFs possess social and/

or ritual values and importance; these also provide the source of feed and nutrition for birds and wild animals.

Hitherto, a total of 255 minor edible fruit yielding species belonging to 149 genera under 61 families, three families to the Liliopsida and 58 families to Magnoliopsida, have been reported from the Bangladesh territory (Pasha and Uddin, 2019). Among these, 48 species are cultivated for fruits, 35 species both cultivated and wild, and the remaining species are exclusively wild. Due to rapid population growth and other anthropogenic activities, alien species, and climate change events – high temperature, change in rainfall pattern, early or late monsoon, frequent floods and cyclones, etc., these valuable plant genetic resources are now declining at an alarming rate. As a result, some of these valuable MF species are now become (critically) endangered and on the verge of extinction. Since its inception in 1963, the Botanical Garden under the Department of Crop Botany, Bangladesh Agricultural University (BAUBG) has been conducting collection and conservation activities. Presently about 1,146 species, more than 20% of the total Bangladesh (Spermatophyte) flora, under 327 genera and 215 families are harboured at this garden (Sarwar, 2019; 2020). Over time, an enormous number of MFs are also conserved in this place. Hitherto, five hundred twenty-seven medicinal and aromatic plant species, many of them are MFs, belonging to one hundred one families have conserved here (Sarwar, 2020). The objective of the present research was to compile an updated checklist of the MF collections of the BAUBG and their conservation priority from national/ international perspectives.

METHODOLOGY

A detailed survey on the MFs growing throughout BAUBG, located at 24°722 463 N 90°442 163 E, was carried out through frequent visits (Sarwar, 2020). During these visits, fresh flowering samples were collected; herbarium specimens were prepared as vouchers by drying the fresh samples properly. The dried specimens were mounted on the herbarium sheet and preserved in Prof. Dr Arshad Ali Herbarium at the Botanical Garden, Department of Crop Botany, Bangladesh Agricultural University. The collected fresh (or dried) specimens were identified in the field or by comparing with herbarium specimens or published literature. The botanical names were updated following <http://www.worldfloraonline.org> (older http://www.theplantlist.org) and their conservation status (and uses) follows "Encyclopedia of Flora and Fauna of Bangladesh" (Ahmed *et al*., 2008a, b, 2009a, b, c, d; Siddiqui *et al*., 2007) and "Red Data Book of Vascular Plants of Bangladesh" (Khan *et al*., 2001; Ara *et al*., 2013). The local names and medicinal uses were documented critically from published literature (Uddin, 2006; Yusuf *et al*., 2009; Uddin *et al*., 2016) and online resources.

RESULTS AND DISCUSSION

A total of 108 species were listed and distributed under 38 families and 67 genera (Table 1; Figs 1-6). Out of these, 30 species are exotic and mostly cultivated; however, a few of them, for example, *Hibiscus sabdariffa*, *Opuntia dellenii*, *Pithecellobium dulce*, *Polyalthia suberosa*, *Tamarindus indica*, etc., have become naturalized and found in the wild also (Pasha and Uddin, 2019). Moreover, Pasha and Uddin (2019) had identified 44 MFs species, many of them encompassed the BAUBG collection, as promising crops for the future due to their taste, colour, wide use and popularity in consumption. Moraceae and Myrtaceae were the most dominant families with 8 taxa each followed by Rutaceae with 7 taxa, Arecaceae, Phyllanthaceae and Sapotaceae with 6 taxa each, and Annonaceae with 5 taxa. The remaining families are represented by 4 or fewer species each (Table 2). Among the genera, *Syzygium* is the largest genus having 7 species (6.48% of the total MFs). *Citrus*, *Ficus* and *Garcinia* come next with 5 species in each genus (4.63%) followed by *Annona*, *Antidesma*, *Ardisia*, *Diospyros*, *Elaeocarpus* and *Flacourtia* with 3 species (2.78%) in each genus (Table 3). These ten genera together account for more than one-third of the total number of MFs the BAUBG. The remaining 11 genera were documented by having 2 species each and 46 genera represented by only 1 species each (Table 1). Among these 108 species, tree species were dominant (85; 78.70%) followed by shrubs (15; 13.89%), herbs (5; 4.63%) and only 3 (2.78%) climber (Fig. 7). The number of recorded MFs (255

Table 1: Minor fruit plant species conserved at the Botanical Garden, Bangladesh Agricultural University. (Ex) Exotic; Clim. Climber; LC Least concern; NE Not evaluated; VU Vulnerable; NT Near threatened; EN Endangered; DD Data deficient; CD Conservation dependent

Sl. No.	Local Name	Botanical Name	Family	Habit	Status	Fig. No.
1.	Bael	*Aegle marmelos* (L.) Corr.	Rutaceae	Tree	LC	1A
2.	Kaju badam	*Anacardium occidentale* L. (Ex)	Anacardiaceae	Tree	LC	1B
3.	Pond apple	*Annona glabra* L. (Ex)	Annonaceae	Tree	NE	1C
4.	Nona-ata	*Annona reticulata* L. (Ex)	Annonaceae	Tree	LC	1D
5.	Sharifa	*Annona squamosa* L. (Ex)	Annonaceae	Tree	LC	1E
6.	Elena/Bignay	*Antidesma acidum* Retz.	Phyllanthaceae	Tree	LC	1F
7.	Choto Sialbuka	*Antidesma bunius* (L.) Spreng.	Phyllanthaceae	Tree	LC	1G
8.	Siyal Buka	*Antidesma montanum* Blume	Phyllanthaceae	Tree	LC	-
9.	Chauldhoa	*Ardisia humilis* Vahl	Primulaceae	Shrub	LC	1H
10.	Bonjami	*Ardisia sanguinolenta* Blume	Primulaceae	Shrub	LC	1I
11.	Bonjam	*Ardisia solanacea* (Poir.) Roxb.	Primulaceae	Shrub	LC	1J
12.	Supari	*Areca catechu* L.	Arecaceae	Tree	LC	1L
13.	Dewa	*Artocarpus lacucha* Buch.-Ham.	Moraceae	Tree	LC	1M
14.	Chapalish	*Artocarpus chama* Buch.-Ham.	Moraceae	Tree	NE	1K
15.	Bilimbi	*Averrhoa bilimbi* L. (Ex)	Oxalidaceae	Tree	LC	1N
16.	Kamranga	*Averrhoa carambola* L. (Ex)	Oxalidaceae	Tree	LC	1O
17.	Latkan	*Baccauria ramiflora* Lour.	Phyllanthaceae	Tree	LC	2A
18.	Tal	*Borassus flabellifer* L	Arecaceae	Tree	LC	2B
19.	Betphal	*Calamus manillensis* L.	Arecaceae	Tree	-	2C
20.	Kumbhi	*Careya arborea* Roxb.	Lecythidaceae	Tree	VU	2D
21.	Karamcha	*Carissa carandas* L. (Ex)	Apocynaceae	Shrub	LC	2E
22.	Bon supari	*Caryota urens* L.	Arecaceae	Tree	LC	2F
23.	Khejur	*Chamaerops humilis* L. (Ex)	Arecaceae	Tree	LC	5C
24.	Star Apple	*Chrysophyllum roxburghii* G. Don (Ex)	Sapotaceae	Tree	LC	-
25.	Ada jamir	*Citrus assamensis* R.M. Dutta & Bhatt.	Rutaceae	Shrub	Rare	2G
26.	Satkora	*Citrus aurantium* L.	Rutaceae	Tree	LC	-
27.	Rough Lemon	*Citrus jambhiri* Lush	Rutaceae	Tree	-	-
28.	Batabi Lebu	*Citrus maxima* (Burm.) Merr.	Rutaceae	Tree	LC	2H
29.	Komla	*Citrus reticulata* Blanco	Rutaceae	Tree	LC	2I
30.	Chalta	*Dillenia indica* L.	Dilleniaceae	Tree	LC	2J
31.	Bon Chalta	*Dillenia pentagyna* Roxb.	Dilleniaceae	Tree	LC	2K
32.	Ashphal	*Dimocarpus longan* Lour.	Sapindaceae	Tree	NT	2L
33.	Beelati Gab	*Diospyros blancoi* A. DC (Ex)	Ebenaceae	Tree	LC	2M
34.	Deshi Gab	*Diospyros malabarica* (Desr.) Kostel	Ebenaceae	Tree	LC	2N
35.	Gulal/Katgula	*Diospyros racemosa* Roxb.	Ebenaceae	Tree	Rare	-
36.	Rudhrakha	*Elaeocarpus angustifolius* Blume	Elaeocarpaceae	Tree	EN	-
37.	Jalpai	*Elaeocarpus floribundus* Blume	Elaeocarpaceae	Tree	LC	2O
38.	Mala	*Elaeocarpus grandiflorus* Sm.	Elaeocarpaceae	Tree	LC	3A
39.	Loquat	*Eriobotrya japonica* (Thunb.) Lindl. (Ex)	Rosaceae	Tree	VU	3B
40.	Surinum cherry	*Eugenia uniflora* L. (Ex)	Myrtaceae	Shrub	-	3C
41.	Makhna	*Euryale ferox* Salisb.	Nymphaeaceae	Herb	NE	3D
42.	Hostikorni dumur	*Ficus auriculata* Lour.	Moraceae	Tree	LC	3E
43.	Fapa-dumur	*Ficus fistulosa* Reinw. *ex* Blume	Moraceae	Tree	Rare	3F
44.	Kak dumur	*Ficus hispida* L.f.	Moraceae	Tree	LC	3G
45.	Jog Dumur	*Ficus racemosa* L.	Moraceae	Tree	NE	3H
46.	Sadimadi dumur	*Ficus semicordata* Buch.-Ham. *ex* Sm.	Moraceae	Tree	Rare	3I

Contd.

Table 1 Contd.

Sl. No.	Local Name	Botanical Name	Family	Habit	Status	Fig. No.
47.	Baichi	*Flacourtia indica* (Burm. f.) Merr.	Salicaceae	Shrub	LC	3J
48.	Tomytomy	*Flacourtia inermis* Roxb.	Salicaceae	Shrub	LC	
49.	Paniala	*Flacourtia jangomas* (Lour.) Raeusch.	Salicaceae	Tree	LC	3K
50.	Kaufal	*Garcinia cowa* Roxb. *ex* DC.	Clusiaceae	Tree	LC	3L
51.	Mangosteen	*Garcinia mangostana* L.	Clusiaceae	Tree	-	
52.	Gutta-gam	*Garcinia morella* (Gaertn.) Desr.	Clusiaceae	Tree	LC	
53.	Thoikar	*Garcinia pedunculata* Roxb. *ex* Buch-Ham.	Clusiaceae	Tree	LC	3M
54.	Dephall	*Garcinia xanthochymus* Hook.f. *ex* Anders.	Clusiaceae	Tree	LC	3N
55.	Phalsa	*Grewia asiatica* L.	Malvaceae	Tree	LC	3O
56.	Raktagota	*Haematocarpus validus* (Miers) Bakh.f. *ex* Forman	Menispermaceae	Clim.	Very Rare	4A
57.	Lal mesta	*Hibiscus sabdariffa* L. (Ex)	Malvaceae	Shrub	NE	-
58.	Bonchalita	*Leea asiatica* (L.) Ridsdale	Leeaceae	Tree	Very Rare	-
59.	Chagal ladi	*Lepisanthes senegalensis* (Poir.) Leenh.	Sapindaceae	Shrub	LC	5O
60.	Kathbel	*Limonia acidissima* L.	Rutaceae	Tree	LC	4B
61.	Mahua	*Madhuca longifolia* (Koenig *ex* L.) MacBr.	Sapotaceae	Tree	NE	3C
62.	Maila-am	*Mangifera longipes* Griff.	Anacardiaceae	Tree	Rare	4D
63.	Uri Aam	*Mangifera sylvatica* Roxb.	Anacardiaceae	Tree	VU	4E
64.	Khirni	*Manilkara hexandra* (Roxb.) Dubard	Sapotaceae	Tree	Rare	-
65.	Sofeda	*Manilkara zapota* (L.) P. *van* Royen (Ex)	Sapotaceae	Tree	LC	4F
66.	Datranga	*Melastoma malabathricum* L. (Ex)	Melastomataceae	shrub	LC	4G
67.	Mainakanta	*Meyna spinosa* Roxb. *ex* Link	Rubiaceae	Shrub	LC	4H
68.	Bakul	*Mimusops elengi* L.	Sapotaceae	Tree	LC	4I
69.	Sajna	*Moringa oleifera* L.	Moringaceae	Tree	LC	4J
70.	Tut	*Morus alba* L. (Ex)	Moraceae	Tree	LC	4K
71.	China cherry	*Muntingia calabura* L.	Muntingiaceae	Tree	-	4L
72.	Paddo, Komol	*Nelumbo nucifera* Gaertn.	Nelumbonaceae	Herb	LC	4M
73.	Phanimanasa	*Opuntia dellenii* Haw. (Ex)	Cactaceae	shrub	LC	4N
74.	Passion fruit	*Passiflora edulis* Sims. (Ex)	Passifloraceae	Clim.	LC	4O
75.	Jhumka lata	*Passiflora foetida* L. (Ex)	Passifloraceae	Clim.	LC	5A
76.	Avocado	*Persea americana* P. Mill. (Ex)	Lauraceae	Tree	CD	-
77.	Khudi Khejur	*Phoenix acaulis* Buch.-Ham. *ex* Roxb.	Arecaceae	Tree	VU	5B
78.	Orboroi	*Phyllanthus acidus* (L.) Skeels.	Phyllanthaceae	Tree	LC	5D
79.	Amloki	*Phyllanthus emblica* L.	Phyllanthaceae	Tree	LC	5E
80.	Khai babla	*Pithecellobium dulce* (Roxb.) Benth.(Ex)	Leguminosae	Tree	LC	-
81.	Murmuri	*Polyalthia suberosa* (Roxb.) Thw. (Ex)	Annonaceae	Tree	Rare	5F
82.	Mock strawberry	*Potentilla indica* (Jacks.) Th.Wolf	Rosaceae	Herb	LC	5G
83.	Gutgutya/Neur	*Protium serratum* (Wall. *ex* Coelbr.) Engl.	Burseraceae	Tree	LC	5H
84.	*Alu Bukhara*	*Prunus bokhariensis* Royle *ex* C.K. Schneid. (Ex)	Rosaceae	Shrub	LC	5I
85.	Buddha Narical	*Pterygota alata* (Roxb.) R.Br.	Malvaceae	Tree	LC	5J
86.	Dalim	*Punica granatum* L. (Ex)	Lythraceae	Tree	LC	5K
87.	Sawtooth blackberry	*Rubus argutus* Link.	Rosaceae	Tree	LC	-
88.	Santol fruit	*Sandoricum koetjape* (Burm.f.) Merr. (Ex)	Meliaceae	Tree	LC	5L
89.	Joyna	*Schleichera oleosa* (Lour.) Merr.	Sapindaceae	Tree	Rare	5M

Contd.

Table 1 Contd.

Sl. No.	Local Name	Botanical Name	Family	Habit	Status	Fig. No.
90.	Choila	*Sonneratia caseolaris* (L.) Engl.	Lythraceae	Tree	NE	5N
91.	Amra	*Spondias pinnata* (L.f.) Kurz	Anacardiaceae	Tree	LC	6A
92.	Beelati Amra	*Spondias purpurea* L. (Ex)	Anacardiaceae	Tree	LC	-
93.	Miracle Fruit	*Synsepalum dulcificum* (Schumach. & Thonn.) Daniell	Sapotaceae	Tree	Rare	6B
94.	Noli Jam	*Syzygium claviflorum*(Roxb.) Wall. *ex* A.M. Cowan & Cowan	Myrtaceae	Tree	LC	6C
95.	Kalo-Jam	*Syzygium cuminii* (L.) Skeels	Myrtaceae	Tree	LC	6D
96.	Khudi jam	*Syzygium cymosum* (Lam.) DC.	Myrtaceae	Tree	NE	6E
97.	Dhaki-jam	*Syzygium grande* (Wight) Walp.	Myrtaceae	Tree	LC	6F
98.	Golab-jam	*Syzygium jambos* (L.) Alston (Ex)	Myrtaceae	Tree	LC	6G
99.	Malay apple	*Syzygium malaccense* (L.) Merr. & Perry (Ex)	Myrtaceae	Tree	LC	6H
100.	Jamrul	*Syzygium samarangense* (Blume) Merr. & Perry (Ex)	Myrtaceae	Tree	LC	6I
101.	Tentul	*Tamarindus indica* L. (Ex)	Leguminosae	Tree	LC	6J
102.	Bohera	*Terminalia bellerica* (Gaertn.) Roxb.	Combretaceae	Tree	LC	6K
103.	Horitoki	*Terminalia chebula* Retz.	Combretaceae	Tree	VU	6L
104.	Kantasingra	*Trapa natans* var. *bispinosa* (Roxb.) Makino	Lythraceae	Herb	LC	6M
105.	Paniphal	*Trapa natans* L.	Lythraceae	Herb	LC	6N
106.	Bonlichu	*Xerospermum noronhianum* (Blume) Blume	Sapindaceae	Tree	Rare	-
107.	Jangli-kul	*Zizyphus glabrata* Heyne *ex* Roth	Rhamnaceae	Tree	EN	-
108.	Jangli Boroi	*Zizyphus oenoplia* (L.) Mill.	Rhamnaceae	Shrub	Rare	6O

taxa) in Bangladesh territory is very smaller compared to the world record (27,400) (French, 2019); therefore, a comprehensive field survey, exploitation, conservation and (large-scale) cultivation, through cultivar development, of MF genetic resources is strongly recommended. Many MF species, also known as "Food for the Poor" (Dandin and Kumar, 2016), are very nutritious, climate-resilient, well adapted to marginal lands and with low-cost inputs, thus may be of great benefit for the survival of poor communities and sustainability of agricultural ecosystems (Saúco, 2008; Krishna *et al*., 2019). The production (and trade) of MFs could play an important role not only in food and nutrition security but also as a source of income (Altendorf, 2018).

Although MFs are commonly used/consumed as sources of micronutrients and phytochemicals – vitamins and minerals, antioxidants, etc., most of these (97 taxa) also have multiple (Ethno-) medicinal uses (Table 4). Along with the treatment of some common diseases e.g., cold, fever, cough, stomachache, asthma, scabies, skin diseases, etc., these MFs are also used for the treatment and/or lowering the risk of some of the deadliest diseases of the world e.g., cardiac problem, diarrhoea and dysentery, diabetes, respiratory tract infection, etc. (https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death). The underutilized MF crops have also vast potential for the production of value-added products, with high therapeutic, medicinal values and antioxidant properties, and free from the residue of toxic chemicals (Krishna *et al*., 2019). Among these species, some MFs e.g., amloki, bael, amrul, bilimbi, horitoki, bohera, kathbel, tentul, ber, kalo-jam/jamun, pomegranate, kamranga, etc., are very common and most popular among the rural people for their medicinal values. Many other fruit species are utilized by the tribal people. Eight MF species were recognized as threatened and/or near-threatened *viz*. endangered 2, vulnerable 5 and near-threatened 1, according to IUCN Red List categories<https://www.iucnredlist.org/>; thirteen

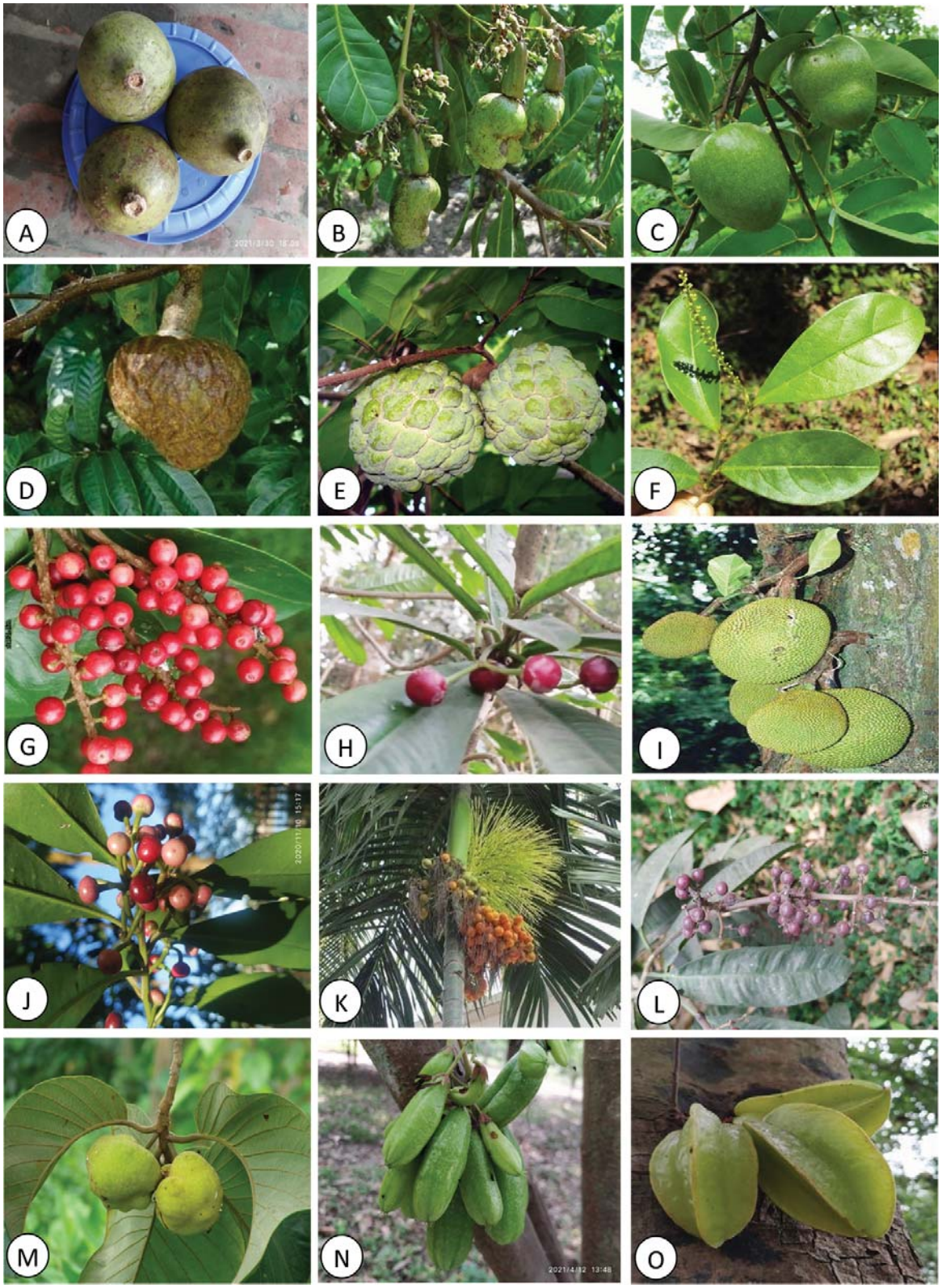

Figure 1: Photographs of minor fruit plants conserved at the Botanic Garden, Bangladesh Agricultural University. A. *Aegle marmelos*; B. *Anacardium occidentale*; C. *Annona glabra*; D. *Annona reticulata*; E. *Annona squamosa*; F. *Antidesma acidum*; G. *Antidesma bunius*; H. *Ardisia humilis*; I. *Ardisia sanguinolenta*; J. *Ardisia solanacea*; K. *Areca catechu*; L. *Artocarpus chama*; M. *Artocarpus lacucha*; N. *Averrhoa bilimbi*; O. *Averrhoa carambola*.

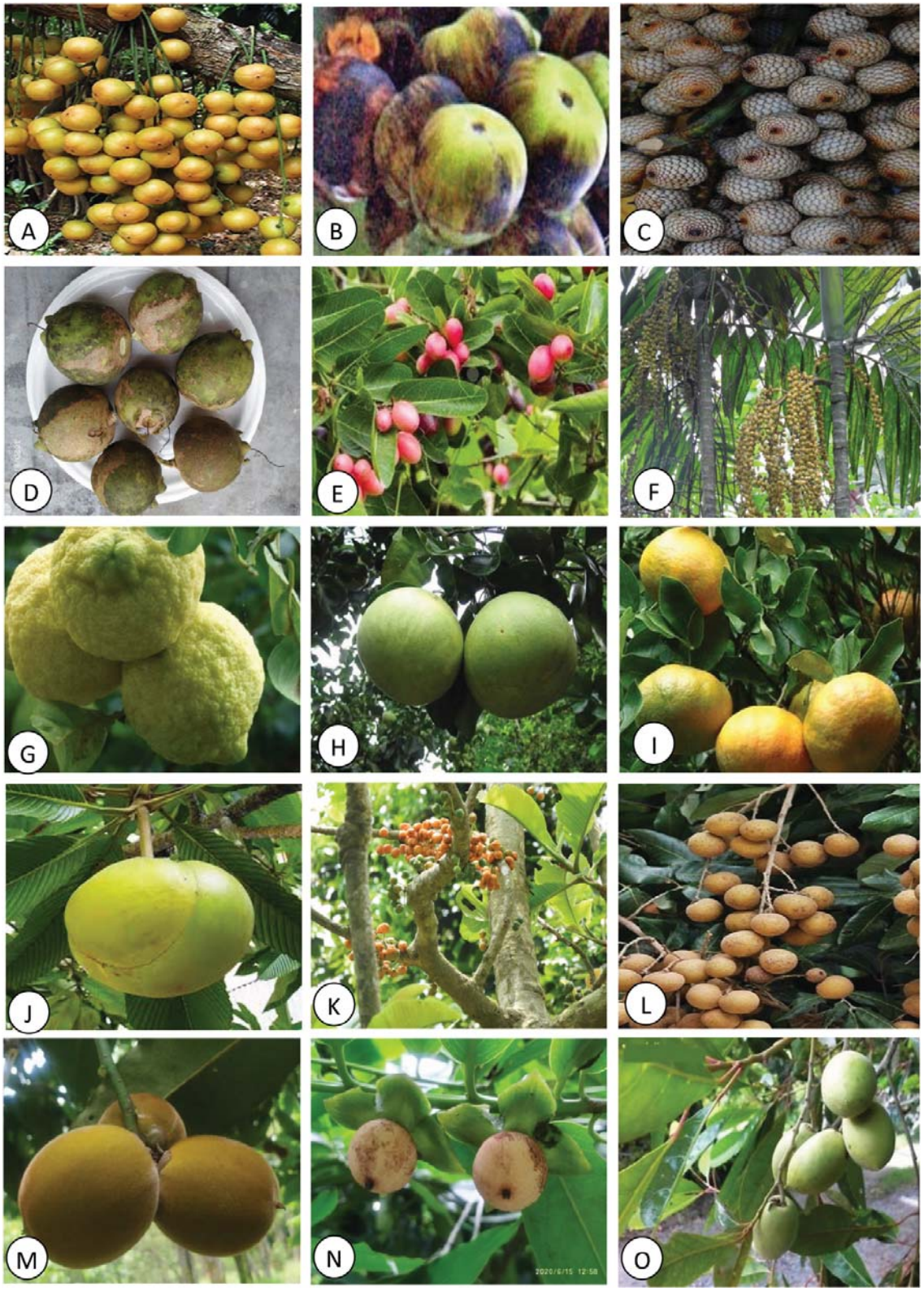

Figure 2: Photographs of minor fruit plants conserved at the Botanic Garden, Bangladesh Agricultural University. A. *Baccauria ramiflora*; B. *Borassus flabellifer*; C. *Calamus manillensis*; D. *Careya arborea*; E. *Carissaa carandas*; F. *Caryota urens*; G. *Citrus assamensis*; H. *Citrus maxima*; I. *Citrus reticulata*; J. *Dillenia indica*; K. *Dillenia pentagyna*; L. *Dimocarpus longan*; M. *Diospyros blancoi*; N. *Diospyros malabarica*; O. *Elaeocarpus floribundus*.

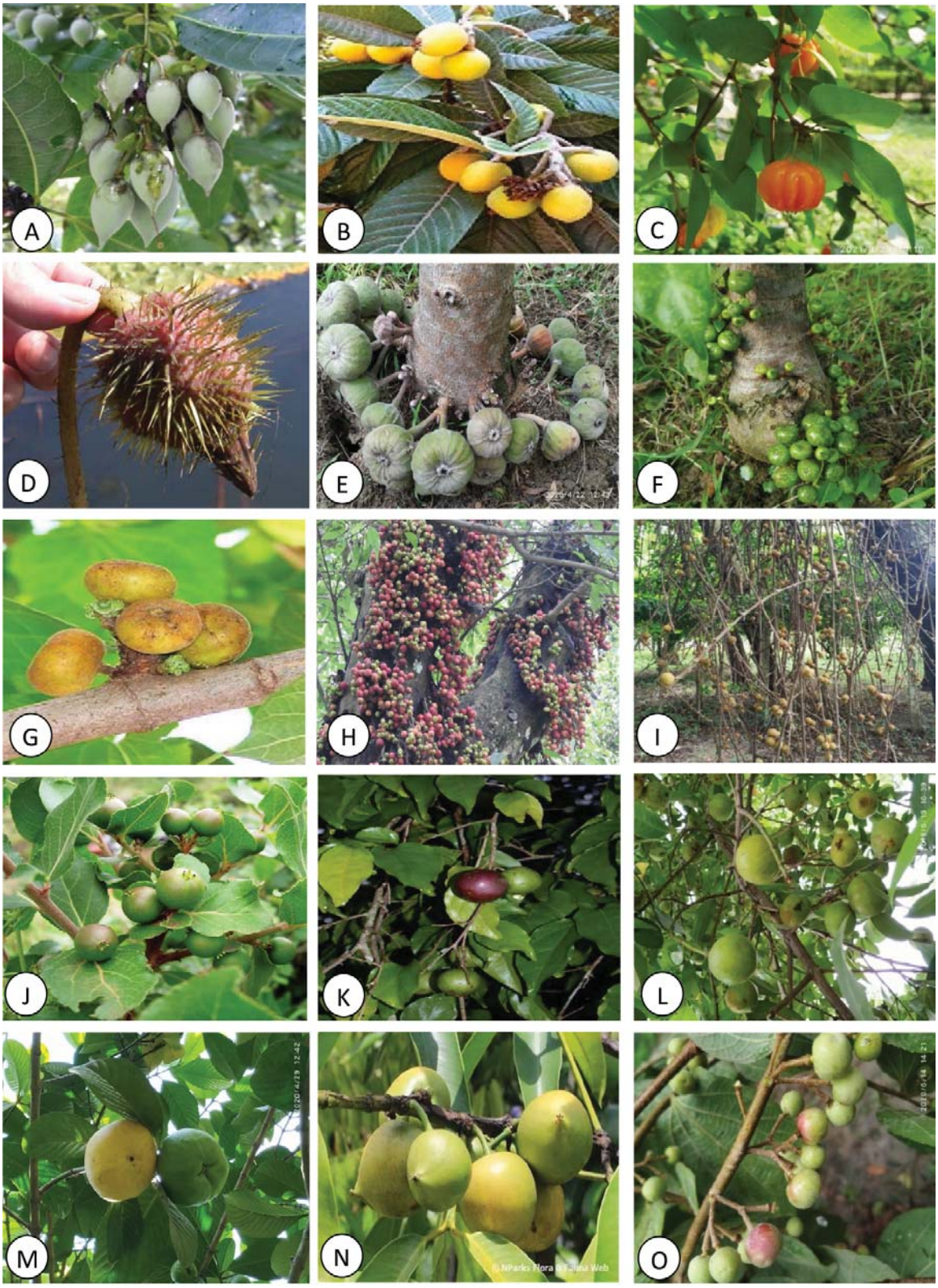

Figure 3: Photographs of minor fruit plants conserved at the Botanic Garden, Bangladesh Agricultural University. A. *Elaeocarpus grandiflorus*; B. *Eriobotrya japonica*; C. *Eugenia uniflora*; D. *Euryale ferox*; E. *Ficus auriculata*; F. *Ficus fistulosa*; G. *Ficus hispida*; H. *Ficus racemosa*; I. *Ficus semicordata*; J. *Flacourtia indica*; K. *Flacourtia jangomas*; L. *Garcinia cowa*; M. *Garcinia pedunculata*; N. *Garcinia xanthochymus*; O. *Grewia asiatica*.

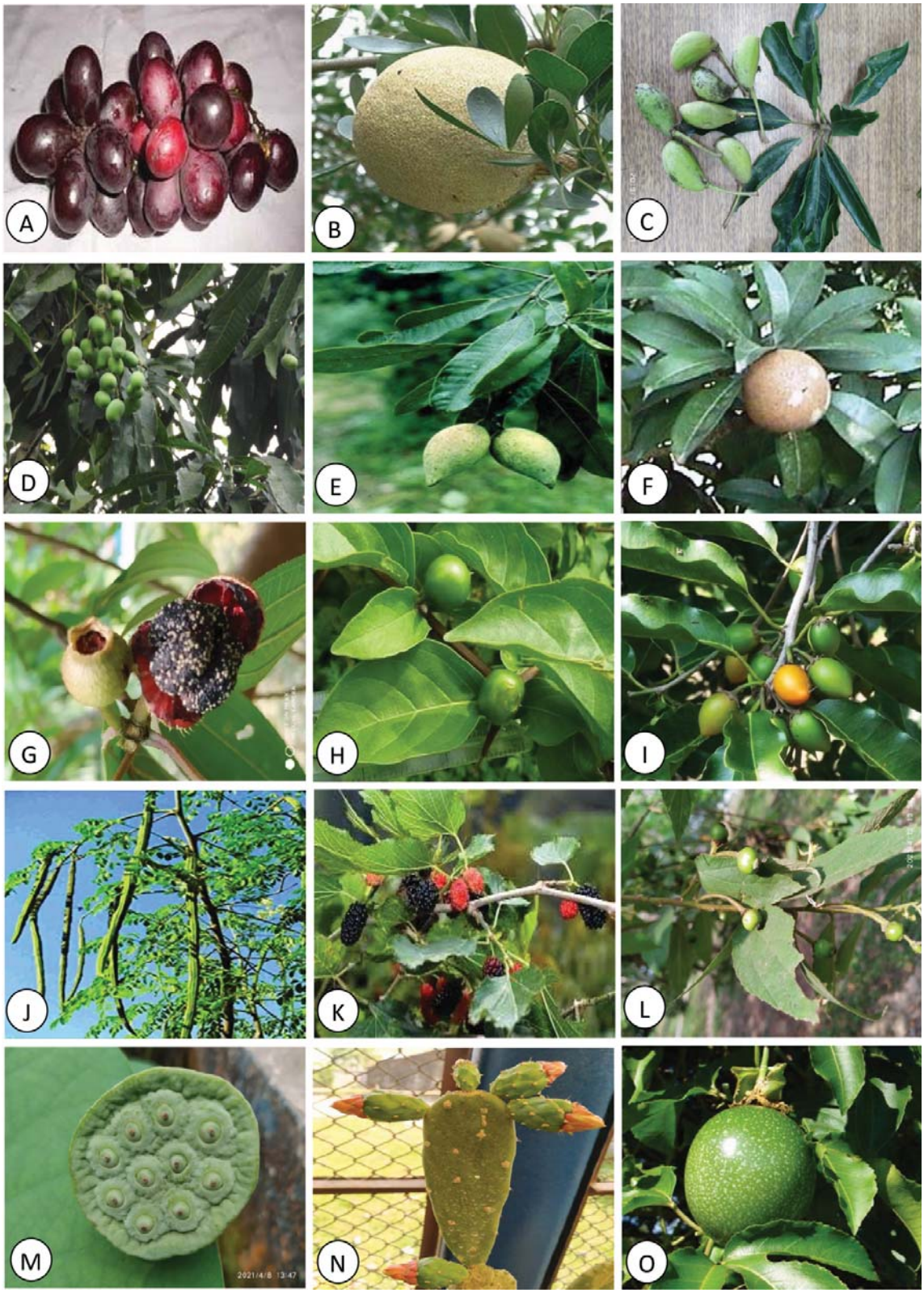

Figure 4: Photographs of minor fruit plants conserved at the Botanic Garden, Bangladesh Agricultural University. A. *Haematocarpus validus*; B. *Limonia acidissima*; C. *Madhuca longifolia*; D. *Mangifera longipes*; E. *Mangifera sylvatica*; F. *Manikara zapota*; G. *Melastoma malabathricum*; H. *Meyna spinosa*; I. *Mimusops elengi*; J. *Moringa oleifera*; K. *Morus alba*; L. *Muntingia calabura*; M. *Nelumbo nucifera*; N. *Opuntia dellenii*; (O) *Passiflora edulis.*

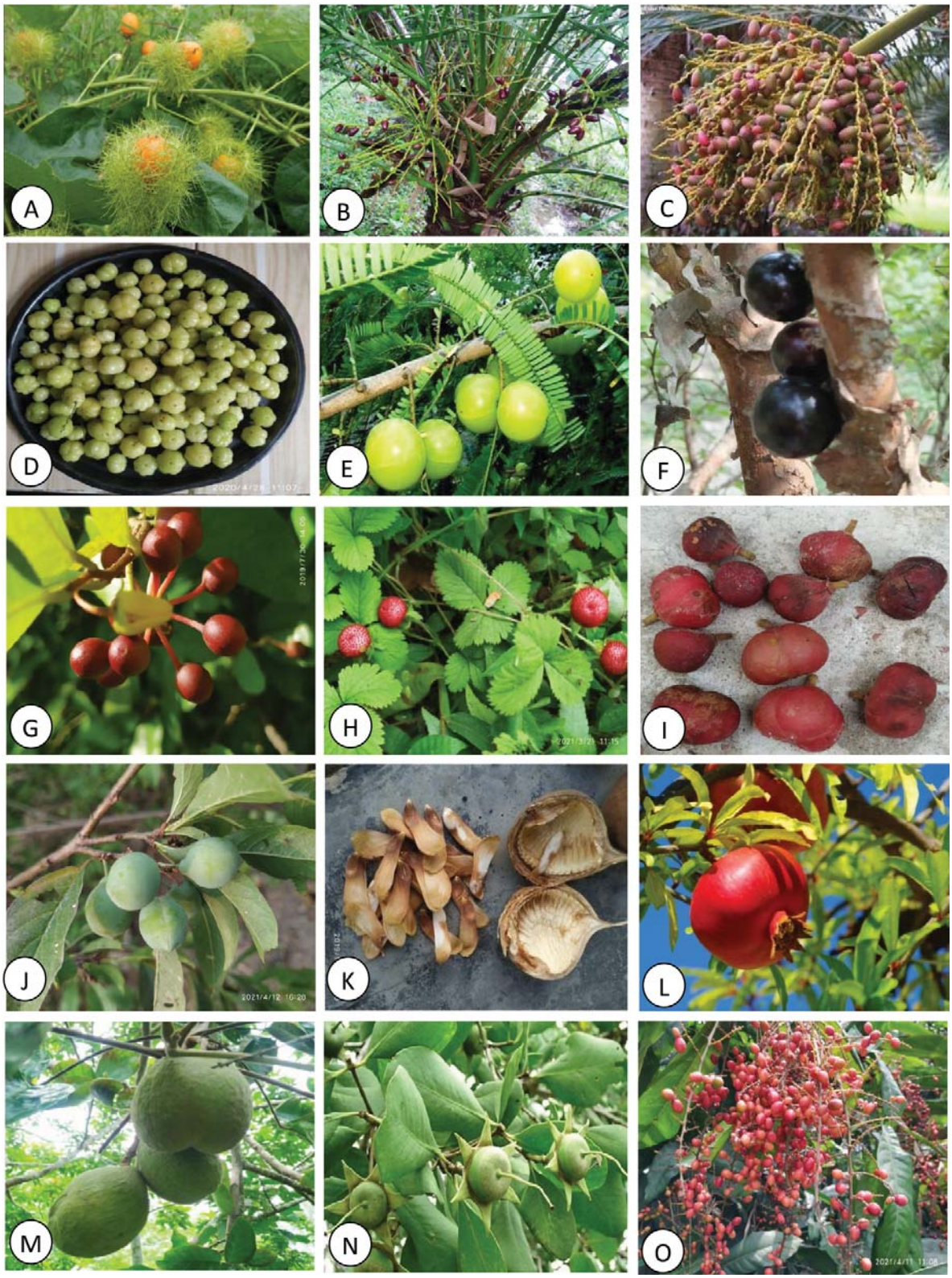

Figure 5: Photographs of minor fruit plants conserved at the Botanic Garden, Bangladesh Agricultural University. A. *Passiflora foetida*; B. *Phoenix acaulis*; C. *Chamaerops humilis*; D. *Phyllanthus acidus*; E. *Phyllanthus emblica*; F. *Plinia cauliflora*; G. *Polyalthia suberosa*; H. *Potentilla indica*; I. *Protium serratum*; J. *Prunus bokhariensis*; K. *Pterygota alata*; L. *Punica granatum*; M. *Sandoricum koetjape*; N. *Sonneratia caseolaris;* O. *Lepisanthes senegalensis*.

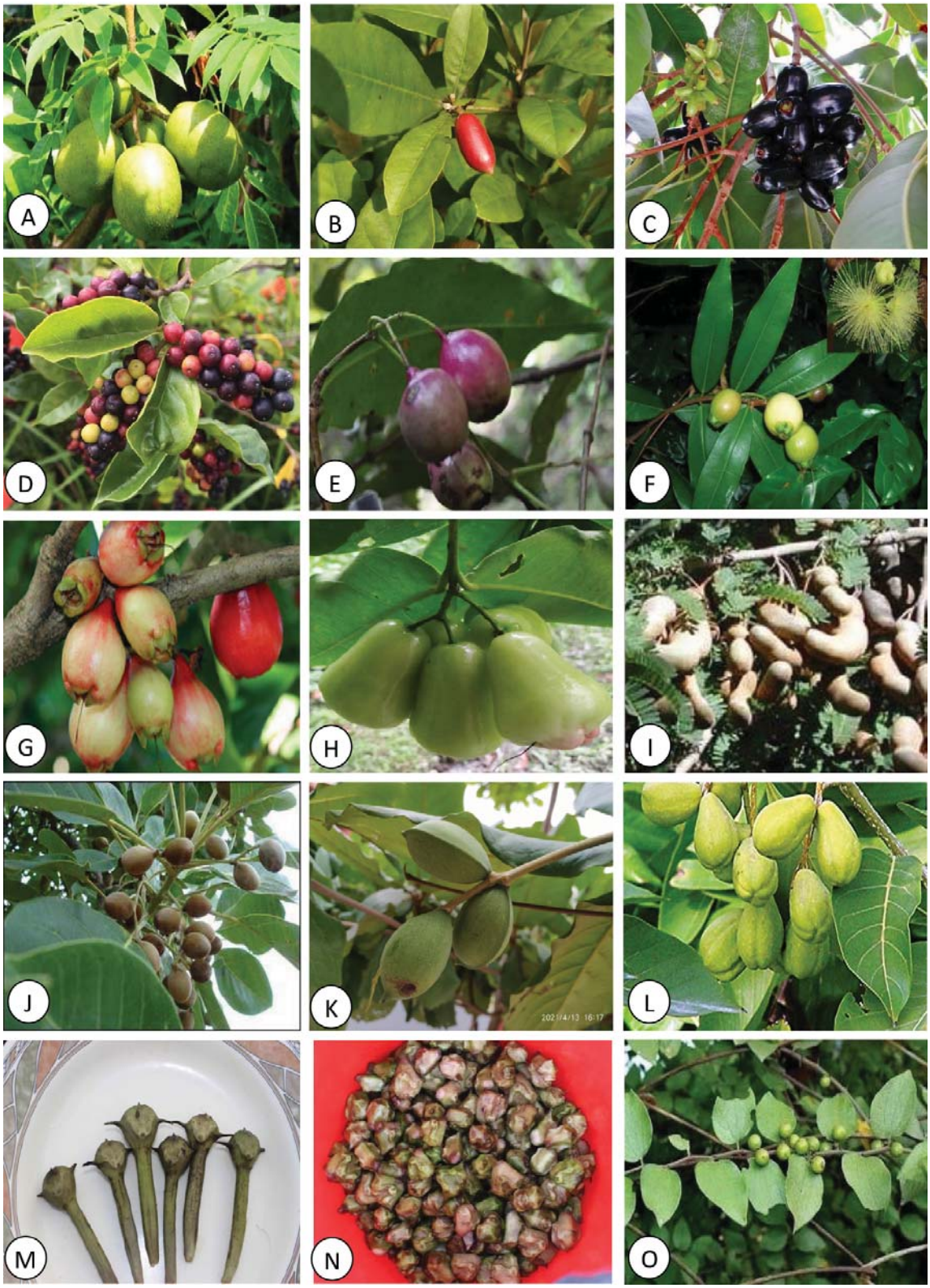

Figure 6: Photographs of minor fruit plants conserved at the Botanic Garden, Bangladesh Agricultural University. A. *Spondias pinnata*; B. *Synsepalum dulcificum*; C. *Syzygium cuminii*; D. *Syzygium cymosum*; E. *Syzygium firmum*; F. *Syzygium jambos*; G. *Syzygium malaccense*; H. *Syzygium samarangense*; I. *Tamarindus indica*; J. *Terminalia bellerica*; K. *Terminalia catappa*; L. *Terminalia chebula*; M. *Trapa natans* var. *bispinosa*; N. *Trapa natans*; O. *Zizyphus oenoplia*.

Table 2: Family-wise distribution of minor fruit taxa at the Botanical Garden, Bangladesh Agricultural University

Sl. No.	Family	Genera	Species	% of taxa in total
1	Anacardiaceae	3	5	4.63
2	Annonaceae	2	4	3.70
3	Apocynaceae	1	1	0.93
4	Arecaceae	6	6	5.56
5	Burseraceae	1	1	0.93
6	Cactaceae	1	1	0.93
7	Clusiaceae	1	5	4.63
8	Combretaceae	1	2	1.85
9	Dilleniaceae	1	2	1.85
10	Ebenaceae	1	3	2.78
11	Elaeocarpaceae	1	3	2.78
12	Lauraceae	1	1	0.93
13	Lecythidaceae	1	1	0.93
14	Leeaceae	1	1	0.93
15	Leguminosae	2	2	1.85
16	Lythraceae	3	4	3.70
17	Malvaceae	3	3	2.78
18	Melastomataceae	1	1	0.93
19	Meliaceae	1	1	0.93
20	Menispermaceae	1	1	0.93
21	Moraceae	3	8	7.40
23	Moringaceae	1	1	0.93
24	Muntingiaceae	1	1	0.93
25	Myrtaceae	2	8	7.41
26	Nelumbonaceae	1	1	0.93
27	Nymphaeaceae	1	1	0.93
28	Oxalidaceae	1	2	1.85
29	Passifloraceae	1	2	1.85
30	Phyllanthaceae	3	6	5.56
31	Primulaceae	1	3	2.78
32	Rhamnaceae	1	2	1.85
33	Rosaceae	4	4	3.70
34	Rubiaceae	1	1	0.93
35	Rutaceae	3	7	6.48
36	Salicaceae	1	3	2.78
37	Sapindaceae	4	4	3.70
38	Sapotaceae	5	6	5.56
	Grand Total	67	108	**100**

species are rare/very rare in the wild. Another institute, the Germplasm Centre (of Fruit Tree Improvement Project), of Bangladesh Agricultural University is also maintaining the germplasms of 67 MFs of Bangladesh (Rahim *et al*., 2011). Only about one-third of MFs (of Bangladesh) were under cultivation (Pasha and Uddin, 2019), others are collected directly from the wild. The reckless collection and over-exploitation from the wild and illegal trade are some of the major drivers of biodiversity losses in the country (Sarwar, 2019). Awareness of the importance and utility of the

Table 3: Top ten species-rich genera of minor fruits at the Botanical Garden, Bangladesh Agricultural University

Genus	No. of species	% in total
Syzygium	7	6.48
Citrus	5	4.63
Ficus	5	4.63
Garcinia	5	4.63
Annona	3	2.78
Antidesma	3	2.78
Ardisia	3	2.78
Diospyros	3	2.78
Elaeocarpus	3	2.78
Flacourtia	3	2.78

underutilized fruit species should be enhanced to encourage and engage the local people/ communities to participate in the conservation efforts. Recently, the Bangladesh Government enhanced the Protected Area (PA) management strategies by recognizing the benefits of collaboration with local communities in their management (DoE, 2015). The co-management, inclusion of local people/beneficiaries in management, of PAs coupled with alternative livelihood opportunities had given impacts biodiversity conservation. Moreover, most of the MF crops are perennial in nature, its cultivation has other benefits for example reduces environmental pollution, improves ecological balance, helps soil and water conservation and also enhances the beauty of the surroundings.

CONCLUSION

Out of the total 255 MF species of Bangladesh, only 108 species are collected and preserved at the BAUBG. The remaining species would be collected and conserved. The conserved MF species might be multiplied and distributed among the rural people and different habitat restoration programmes. The development of improved cultivars and new value-added products and/or by-products, and the expansion of the planting area of these MF species hold great potentials for improving nutrition and food security in Bangladesh and the world as well.

ACKNOWLEDGEMENTS

(Late) Prof. Dr M. Arshad Ali, the founder Curator, Prof. M. Mustafizur Rahman and the Present and previous Curators who have enriched the collection and curated the plant genetic resources of this Botanical Garden are thankfully acknowledged.

REFERENCES :

Ahmed, Z.U., Begum, Z.N.T., Hassan, M.A., Khondker, M., Kabir, S.M.H., Ahmad, M., Ahmed, A.T.A., Rahman, A.K.A. and Haque, E.U. (eds.) 2008a. *Encyclopedia of Flora and Fauna of Bangladesh, Vol. 6. Angiosperms: Dicotyledons (Acanthaceae-Asteraceae)*. Asiatic Society of Bangladesh, Dhaka. pp. 1-408.

Ahmed, Z.U., Hassan, M.A., Begum, Z.N.T., Khondker, M., Kabir, S.M.H., Ahmad, M., Ahmed, A.T.A., Rahman, M.A.K. and Haque, E.U. (eds.) 2008b. *Encyclopedia of Flora and Fauna of Bangladesh, Vol. 12. Angiosperms: Monocotyledons (Orchidaceae-Zingiberaceae)*. Asiatic Society of Bangladesh, Dhaka. pp. 1-552.

Ahmed, Z.U., Hassan, M.A., Begum, Z.N.T., Khondker, M., Kabir, S.M.H., Ahmad, M. and Ahmed, A.T.A. (eds.) 2009a. *Encyclopedia of Flora and Fauna of Bangladesh, Vol. 7. Angiosperms: Dicotyledons (Balsaminaceae-Euphorbiaceae)*. Asiatic Society of Bangladesh, Dhaka. pp. 1-546.

Table 4: Medicinal uses of minor fruit plants at the Botanical Garden, Bangladesh Agricultural University

Sl. No.	Local Name	Parts used	Medicinal Uses
1.	Ada jamir	Fruits	Laxative, appetizer, stomachic, digestive, anthelmintic, dyspepsia, flatulence, helminthiasis, cold fevers, sore throats, sinusitis, bronchitis, asthma, antidepressant, rheumatism arthritis, obesity, an astringent, herpes, cuts, insect bites
2.	*Alu Bukhara*	Seed and young shoots	Toxic
3.	Amloki	Fruits, fresh bark	Jaundice, dyspepsia & coughs, cardiac problem, nasal congestion, retention of urine, diabetes, eye diseases & high cholesterol
4.	Amra	Unripe fruits	Diabetes, heart ailment & urinary troubles
5.	Amrul	Fruits and bark	Tuberculosis, mouth infection, stomach ache and abdominal ailments, cough, yellow urine, bad appetite, deep bone pains, diabetes, gonorrhoea, swollen stomach after childbirth, sore throat, bronchitis, constipation
6.	Ashphal	Fruits	Anti-depressant, neurasthenic neurosis and insomnia, reduce the risk of cancer, improve blood circulation, prevents anaemia, reduce the risk of cardiac arrest & strokes
7.	Avocado	Leaves, bark, fruit, seed	Dysentery, coughs, high blood pressure, liver problems, gout, diarrhoea, blood cholesterol, promote hair growth, smooth skin and treat skin conditions, aphrodisiac
8.	Bael	Ripe fruits	Fever, catarrh or the inflammation of the mucous membrane and asthma, melancholia or a depressed unhappy emotional state, scurvy, chronic diarrhoea & irritation of the alimentary tract
9.	Baichi	Ripe fruits, leaves, roots, bark	Snakebite, malaria, diarrhoea, pneumonia, intestinal worms, arthritis jaundice & enlarged spleens
10.	Bakul	Fruits, leaves and bark, flowers	Diarrhoea & dysentery, gum inflammation, toothache, gonorrhoea, snakebites, fever, headache
11.	Batabi Lebu	Leaves, fruits and seeds, flowers	Coughs, fevers, gastric disorders
12.	Beelati Amra	Fruits and leaves	Swollen glands & trauma, headache, constipation, dysentery & diarrhoea
13.	Beelati Gab	Fruits, leaves, bark	Cough & stomach ache, fever & also skin diseases
14.	Betphal		Stomach problems including ulcers, inflammation of the stomach lining, diarrhoea, intestinal gas, upset stomach
15.	Bilimbi	Fruits, flowers, leaves	Applied as a paste or itches, swelling of mumps & rheumatism & on skin eruptions, venereal diseases, coughs, beriberi & biliousness, fever, inflammation, rectal bleeding, internal haemorrhoids
16.	Bohera	Fruits	Diarrhoea and indigestion, chronic constipation, upper respiratory tract infections
17.	Bon chalta	Unripe fruits	Vata, khapa, anal fistula, wounds, diabetes, diabetic carbuncle, neuritis, pleurisy, pneumonia, burning sensation
18.	Bon Khejur	Ripe fruits, sap	Heart ailments, abdominal complaints, fever, vomiting and loss of consciousness, gonorrhoea
19.	Bon supari	Seed, root, bark, flower	Gastric ulcers, migraine headaches, snake-bite poisoning and rheumatic swellings, tooth ailments, boils, promoting hair growth
20.	Bonchalita	Tender shoots, leaves, roots, fruits	Guinea worms, snake-bite
21.	Bonjam	Roots, bark	Fever, dropsy, diarrhoea, rheumatism, concussion or bruises, indigestion
22.	Bonjami	Root, leaves, fruits, whole plant	Diarrhoea, cough, rheumatism or lumbago, colic, gonorrhoea, antileprotic, liver diseases, fever
23.	Bonlichu	Fruits, leaves	Stomach-ache
24.	Buddha Narical	Fruit, bark	Narcotic, haemorrhoids, dropsy, swelling oedema, gout, leprosy, pain
25.	Chagal ladi	Whole plant, seeds, flowers, roots	Epilepsy, mental illness, hemicranias, jaundice, hepatoralgia, cough, gastropathy, hernia, haemorrhoids, helminthiasis, dyspepsia & skin diseases
26.	Chalta	Unripe fruits	Vata & kapha disorders, fatigue, abdominal pains, regulate the heat of the body & tone up the nervous system.
27.	Chauldhoa	Fruit, leaves	Cancer, heart diseases, liver poisoning, scabies
28.	Choila	Plant, leaves, ripe fruits	Haemostatic, sprains, swellings, worms, coughs, make poultices onto cuts and bruises, haematuria, smallpox
29.	Choto Sialbuka	Fruits, leaves	Heart diseases, high blood pressure, coughs, indigestion, syphilis, gonorrhoea
30.	Dalim	Ripe fruits	Diarrhoea, dysentery & intestinal parasites, nose bleeding, gum bleeding.
31.	Datranga	Leaves, shoots, barks, seeds,	Diarrhoea, dysentery, haemorrhoids, cuts and wounds, toothache, stomachache, antinociceptive, anti-inflammatory,

Sl. No.	Local Name	Parts used	Medicinal Uses
		and roots	wound healing
32.	Dephall	Ripe fruits	Anorexia, biliousness, dysentery, jaundice, malaria, pain, phlegm.
33.	Deshi Gab	Ripe fruits	Heal sores & wounds, diarrhoea & dysentery, blood disease, gonorrhoea & leprosy, bilious fever, tumours
34.	Dewa	Ripe fruits	Pimples, cuts and wounds, skin aliments, headache
35.	Elena	Leaves, roots, ripe fruits	Dysentery & bile complaints, dropsy, muscular pains, pneumonia, sores
36.	Fapa-dumur	Root, leaves	Diaphoretic, post-natal treatment, narcotic
37.	Goda	Root, bark	Astringent, anthelmintic, gastrointestinal disorders
38.	Golab-jam	Ripe fruits	Diarrhoea, dysentery and catarrh, diabetes, asthma, bronchitis and hoarseness, sore eyes, rheumatism, epilepsy
39.	Gutgutya	Ripe fruits	Mouth ulcers
40.	Gutta-gam	Fruits	Dysentery, gastritis, anti-inflammatory
41.	Horitoki	Fruits, bark, leaves	Leprosy, anaemia, narcosis, fever, piles, heart diseases, diarrhoea, anorexia
42.	Hostikorni dumur	Stem latex, fruits	Cuts, wounds, cuts, wounds
43.	Jalpai	Leaves, bark, fruits	Diarrhoea. Juice is very good for quenching thirst for diabetic patient
44.	Jamrul (Thai)	Young leaves, shoots, flowers, fruits	Tuberculosis, mouth infections, stomach ache and abdominal ailments, red eyes, skin infections, cough, yellow urine, bad appetite, deep bone pains, diabetes
45.	Jangli boroi	Leaves, bark, ripe fruits	Wounds, mouthwash for sore throats, dysentery, inflammation of the uterus
46.	Jhumka lata	Leaves, stem	Sleeping problems, itching, cough, anthelmintic, for intestinal nematodes and flatworms, colds, chest coughs, tuberculosis, worms, improve fertility in women, antispasmodic, snake bite
47.	Jog dumur	Ripe fruits	Diabetes, liver disorders, diarrhoea, inflammatory conditions, haemorrhoids, respiratory, urinary diseases
48.	Joyna	Bark, seed, fruit	Itching, acne, burns, other skin troubles, rheumatism (external massage), hairdressing, hair growth, astringent, leprotic ruptures, skin inflammations, ulcers, malaria
49.	Kaju-badam	Peduncle, cotyledons, bark, nut	Tooth abscesses, diarrhoea, snake bites, antifungal agent, cracked heels
50.	Kak dumur	Roots, bark, fruits	Fevers, antiperiodic, emetic, tonic, emetic, liver problems,ulcers, psoriasis, anemia, piles, jaundice, vitiligo, hemorrhage, diabetes, convulsion, hepatitis, dysentery, biliousness, lactagogue, purgative
51.	Kalo-Jam	Ripe fruits	Source of iron, diabetes, heart and liver troubles, quenching thirst for diabetic patients
52.	Kamranga	Leaves, flowers, fruits	Antimicrobial activity against *E. coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Bacillus cereus*
53.	Kantasingra	Fruit	Stomach problems, genitourinary system, liver, kidney, spleen, astringent, stomachic, diuretic, febrifuge, antiseptic
54.	Karamcha	Ripe & unripe fruits	Rich source of iron and vitamin C, antiscorbutic, anaemia
55.	Kathbel	Ripe fruits	Diarrhoea, dysentery, hiccough, sore throat, snakebite, biliousness, intestinal troubles of children
56.	Kaufal	Fruit, fruit rind, leaves, bark	Cramps, dysentery, headache, nausea, stomach ache, vomiting
57.	Khaia Babla	Bark, pulp, leaves, seed	Astringent, gum ailments, toothache and bleeding,chronic diarrhoea, dysentery, constipation, tuberculosis, spontaneous abortion, gall bladder ailments, wounds, ulcers
58.	Khejur	Ripe fruits, sap	Worms, heart ailments, fever, stomach pain, etc.
59.	Khirni	Fruits and bark, flowers	Dental ailments, wound and dysentery, disease of gums, blood diseases
60.	Khudi jam	Ripe fruits	Antimalarial activity
61.	Komla	Fruit and fruit rind	Dyspepsia, gastro-intestinal distension, cough with profuse phlegm, hiccup and vomiting, hernia, lumbago, mastitis, pain on swelling of the testes
62.	Kumbhi	Fruit, bark, leaves	Body swelling, cough and colds
63.	Lal mesta	Fruit, leaves	Laxative, increase urination, feet cracks, bilious, sores, wounds, sour throat, wounds healing, antimicrobial, emollient, antipyretic, diuretic, anti-helmentic, sedative, cough poultice on abscesses
64.	Latkan	Bark	Eye inflammation, sore eyes
65.	Loquat	Fruits	Bronchitis, cough, feverish colds, allaying vomiting, thirst

Sl. No.	Local Name	Parts used	Medicinal Uses
66.	Mahua	Flowers, Ripe fruits	Bleeding gums and ulcers, diabetes, neurotic disorder, heart diseases, cough, ear troubles and bronchitis
67.	Mainakanta	Fruits, leaves	Phlegm & bile, diphtheria
68.	Makhna	Fruits, young stalks, rhizomes	Chronic diarrhoea, vaginal discharge, kidney weakness
69.	Mangosteen	Fruits	Abdominal pain, diarrhoea, dysentery, infected wound, suppuration, chronic ulcer
70.	Miracle fruit	Root, leaves, bark	Diabetic, blood cholesterol-lowering, anti-hyperuricaemia, antioxidant, anticonvulsant, anticancer, malaria, hyperthermia, prostate ailments, gonorrhoea, asthma, male infertility, weight loss
71.	Mock strawberry	Plant, leaves	Anticoagulant, antiseptic, depurative, febrifuge, diarrhoea, digestive upsets, gout, laryngitis, acute tonsillitis and as a gargle for sore throats
72.	Murmuri	Fresh root, bark	Abortifacient, abdominal pain, febrifuge analgesic, laxative
73.	Nona-ata	Ripe fruits	Worms, abscesses and ulcers, diarrhoea and dysentery.
74.	Orboroi	Fruits, leaves	Asthma, cure skin diseases, itching, fever, urticaria, treat bronchial catarrh, enrich the blood
75.	Paddo	Rhizomes, leaves, seeds	Hematemesis, epistaxis, hematuria, lowering blood sugar levels, diarrhoea, cholera, fever, hyperdipsia
76.	Paniala	Fruit, young shoots	Jaundice, enlarged spleens, diarrhoea and dysentery, malaria, snakebites
77.	Paniphal	Fruit	Elephantiasis, pestilent fevers, rheumatism, sores, sunburn, skin complaints
78.	Passion fruit	Fruit, flower	Mild sedative, bronchial asthma, insomnia, nervous gastrointestinal disorders, menopausal problems, rheumatism or gout, diuretic, inhibition of the cancer cell growth
79.	Phalsa	Fruits	Pustular eruptions, rheumatism
80.	Phanimanasa	Fruit, leaves	Anti-inflammatory, anti-oxidant, antidiabetic activity, immunomodulatory effect, anti-depressant, hypotensive, in acute liver injury, low-density lipoprotein peroxidation, anti-hyperlipidemia
81.	Pond apple	Bark, leaves	Flatworms, nematodes, sedative, cardiotonic infusion, anticancer
82.	Raktagota	Fruits, leaves	Sore throat, inflamed tonsils, liver
83.	Rough lemon	Fruits, leaves	Arthritis, digestive disorders, analgesic, anti-inflammatory, antioxidant, anthelmintic, antibacterial, antifungal, hypolipidemic. antihyperglycemic, antidiabetic, hypoglycemic activity
84.	Rudhrakha	Fruit, seeds, bark	Poison antidote, mental diseases, epilepsy, asthma, hypertension, arthritis, liver diseases, blood pressure, heart ailments, stomach-ache, chest and shoulders pain, enlarged spleen
85.	Sadimadi dumur	Roots, bark, fruits	Headaches, fevers, menstrual disorders, gastric troubles, peptic ulcers, constipation
86.	Sajna	Leaves, roots, seed, bark, fruit, flowers, immature pods	Cardiac and circulatory stimulants, possess antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, diuretic, antispasmodic, antihypertensive, cholesterol-lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities
87.	Santol	Leaves, Bark, roots	Fever, diarrhoea, tonic after childbirth, ringworm, carminative, dysentery, anti-cancer activity, anti-spasmodic, carminative, antiseptic, astringent, stomachic
88.	Satkora	Fruit	Vasoconstriction, elevation of blood pressure, relaxation of bronchial muscle, gastrointestinal disorders, insomnia, headaches, cardiovascular diseases, cancer, antiseptic, antioxidant, antispasmodic, aromatic, astringent, carminative, digestive, sedative, stimulant, stomachic, tonic
89.	Sharifa	Root, leaves, seed	Analgesic, anti-inflammatory, anti-microbial, cytotoxic, anti-lipidimic, anti-ulcer, molluscicidal properties, genotoxic, vasorelaxant, anti-tumour, hepatoprotective, larvicidal, insecticidal, anthelmintic
90.	Siyal Buka	Fruit, leaves, roots and stem	Measles, chickenpox, malaria, headache, thrush in children, diuretic
91.	Sofeda	Ripe fruits	Diarrhoea, relieve pulmonary complaints, coughs, colds, expel bladder and kidney stones
92.	Star Apple	Fruits, roots, leaves	Inflammation in laryngitis and pneumonia, diabetes mellitus, to relieve angina, intestinal disturbance
93.	Supari	Seed	Hunger, abdominal discomfort, anaemia, leucoderma, leprosy, obesity
94.	Tal	Fruits, endosperm, latex	Biliousness, dysentery, gonorrhoea, liver disorders, ulcers, heartburn, enlarged spleen & liver
95.	Tentul	Ripe and unripe fruits	Stomach disorders, general body pain, jaundice, yellow fever, blood tonic, skin cleanser, antiseptic, scurvy, cough
96.	Thoikar	Ripe and unripe fruits	Carminative, unripe angina pectoris, abdominal tumour, haemorrhoids, to allay thirst, biliousness, constipation, heartburn; appetizer; gains weight
97.	Tut	Ripe fruits, root	Leukaemia, hyperuricemia & gout, diabetes

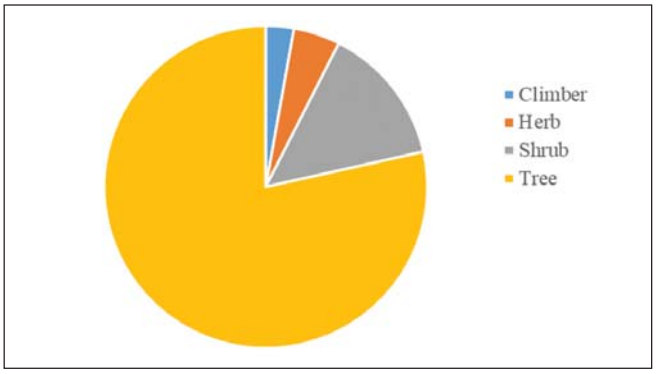

Fig. 7: Growth habit of minor fruits at Botanical Garden, Bangladesh Agricultural University

Ahmed, Z.U., Hassan, M.A., Begum, Z.N.T., Khondker, M., Kabir, S.M.H., Ahmad, M. and Ahmed, A.T.A. (eds.) 2009b. *Encyclopedia of Flora and Fauna of Bangladesh, Vol. 8. Angiosperms: Dicotyledons (Fabaceae-Lythraceae)*. Asiatic Society of Bangladesh, Dhaka. pp. 1-474.

Ahmed, Z.U., Hassan, M.A., Begum, Z.N.T., Khondker, M., Kabir, S.M.H., Ahmad, M. and Ahmed, A.T.A. (eds.) 2009c. *Encyclopedia of Flora and Fauna of Bangladesh, Vol. 9. Angiosperms: Dicotyledons (Magnoliaceae-Punicaceae)*. Asiatic Society of Bangladesh, Dhaka. pp. 1-488.

Ahmed, Z.U., Hassan, M.A., Begum, Z.N.T., Khondker, M., Kabir, S.M.H., Ahmad, M. and Ahmed, A.T.A. (eds.) 2009d. *Encyclopedia of Flora and Fauna of Bangladesh, Vol. 10. Angiosperms: Dicotyledons (Ranunculaceae-Zygophyllaceae)*. Asiatic Society of Bangladesh, Dhaka. pp. 1-580.

Altendorf, S. 2018. Minor tropical fruits – Main streaming a niche market. *Food Outlook July 2018*. FAO. pp. 67-75.

Ara, H., Khan, B. and Uddin, S.N. (eds.) 2013. *Red Data Book of Vascular Plants of Bangladesh*, Vol. 2. Bangladesh National Herbarium, Dhaka. pp. 1-280.

Dandin, S.B. and Kumar, N.K.K. 2016. *Neglected and underutilized fruit species – an insurance against global mal and under nutrition*. Regional Expert Consul., 3-5 December 2016, FAO Regional Office, Bangkok.

DoE (Department of Environment). 2015. *Fifth National Report to the Convention on Biological Diversity*. Min. Environ. & Forest, Govt. of Bangladesh, Dhaka, pp. 1–164.

French, B. 2019. Food Plants International database of edible plants of the world, a free resource for all. In: Ravindran C. *et al*. (eds.) *Proc. III Int. Symp. Underutilized Plant Species. Acta Hortic.*, **1241**: 1-6.

Ghosh, S.N. (ed.) 2017. *Minor Fruits: Nutraceutical Importance and Cultivation* (3 Parts). Jaya Pub. House, Delhi.

Khan, M.S., Rahman, M.M. and Ali, M.A. (eds.) 2001. *Red Data Book of Vascular Plants of Bangladesh*. Bangladesh National Herbarium, Dhaka. pp. 1-140.

Krishna, H., Saroj, P.L., Maheshwari, S.K., Singh, R.S., Meena, R.K., Chandra, R. and Parashar, A. 2019. Underutilized fruits of arid & semi-arid regions for nutritional and livelihood security. *Int. J. Minor Fruits Med. Aroma. Plants*, **5**(2): 1-14.

Pasha, M.K. and Uddin, S.B. 2019. Minor edible fruits of Bangladesh. *Bangladesh J. Plant Taxon.*, **26**: 299-313.

Rahim, M.A., Alam, A.K.M.A., Alam, M.S. and Anwar, M.M. (eds.) 2011. *Underutilized Fruits in Bangladesh*. Bangladesh Agriculture University, Mymensingh, pp. 1-205.

Roya, A. and Bauri, F.K. 2019. Scope of minor fruit production in India. In: Ravindran C. *et al*. (eds.) *Proc. III Int. Symp. Underutilized Plant Species. Acta Hortic.*, **1241**: 43-49.

Sarwar, A.K.M. Golam. 2019. Plant Biodiversity and Conservation of Higher Plants in Bangladesh: Present Status and Future Prospects. In: Sangeetha, J., Thangadurai, D., Goh, H.C. and Islam, S. (eds.) *Biodiversity and Conservation: Characterization and*

Utilization of Plants, Microbes and Natural Resources for Sustainable Development and Ecosystem Management. AAP/CRC, Florida, USA. pp. 259-288**.**

Sarwar, A.K.M. Golam. 2020. Medicinal and aromatic plant genetic resources of Bangladesh and their conservation at the Botanical Garden, Bangladesh Agricultural University. *Int. J. Minor Fruits Med. Aroma. Plants*, **6**(2): 13-19.

Saúco, V.G. 2008. Global overview of underutilized tropical and subtropical fruits. In: Oh, D.-G. and Kubota C. (eds.) *Proc. XXVII IHC – Cultiv. Utiliz. Asian, Sub-Trop. Underutilized Hort. Crops*. *Acta Hort.*, **770**: 77-85.

Siddiqui, K.U., Islam, M.A., Ahmed, Z.U., Begum, Z.N.T., Hassan, M.A., Khondker, M., Rahman, M. M., Kabir, S.M.H., Ahmad, M., Ahmed, A.T.A., Rahman, A.K.A. and Haque, E.U. (eds.) 2007. *Encyclopedia of Flora and Fauna of Bangladesh, Vol. 11. Angiosperms: Monocotyledons (Agavaceae-Najadaceae)*. Asiatic Society of Bangladesh, Dhaka. pp. 1-399.

Srivastava, A., Bishnoi, S.K. and Sarkar, P.K. 2017. Value Addition in Minor Fruits of Eastern India: An Opportunity to Generate Rural Employment. In: Dutta, A.K. and Mondal, B. (eds.) *Fruits for Livelihood: Production Technology and Management Practices*. Agrobios (India), Jodhpur, India, pp. 395–417.

Uddin, M.S., Uddin, S.B. and Lee, S.W. 2016. *Ethnomedicinal Plants of Bangladesh*. International Biological Material Research Center (IBMRC), Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon, Republic of Korea. 204 pp.

Uddin, S.N. 2006. *Traditional Uses of Ethnomedicinal Plants of the Chittagong Hill Tracts*. Rahman, M.M. (ed.), Bangladesh National Herbarium, Mirpur, Dhaka.

Yusuf, M., Begum, J., Hoque, M.N. and Chowdhury, J.U. 2009. *Medicinal Plants of Bangladesh*. 2nd Rev. Enlarg. ed., Bangladesh Council of Scientific and Industrial Research Lab, Chittagong. 340 pp.

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The protective effect of Crataegus monogyna Jacq aqueous extract (fruits and leaves) on blood cells and lipid profile of rats after copper induced-toxicity

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ABSTRACT

The objective of this work is to use the Hawthorn Crataegus monogyna, as a protective agent against copper chronic intoxication. Male Wistar rats were divided into six groups; the control received tap water, standard diet ad libitum, two positive controls treated respectively with Hawthorn leaves and fruits aqueous extract, a group treated with Cu and finally, two groups treated with Cu+leaves (CuL) and Cu+fruits (CuF). The treatment was done by gavage for 30 consecutive days, where: glucose-6-phosphate of erythrocytes (G6PD), white blood cells (WBC), red blood cells (RBC), platelets (PLT), hemoglobin (HGB), hematocrits (HCT), mean corpuscular volume (MCV), triglycerides (TRIG), cholesterol (CHOL), high density lipoproteins (HDL) and low density lipoproteins (LDL) were measured. Copper treatment reduced G6PD, RBC, HGB, HCT, MCV, TRIG and CHOL levels, compared to the control. Compared to the Cu group, the two combined treatments (Cu L and Cu F) have an increase on G6PDH, RBC, HGB, HCT, MCV, TRIG and CHOL levels, with a decrease in WBC, PLT, and LDL levels. As a conclusion, hawthorn aqueous extracts have mitigated copper toxicity towards blood cells and LDL of wistar rats.

Keywords **:** Glucose-6-Phosphate Dehydrogenase, hawthorn, high density lipoprotein, red blood cells

INTRODUCTION

Copper is a trace element essential for many biological processes, but it becomes harmful when it exceeds the threshold level (Abbas *et al.,* 2018). About 60% of consumed copper is absorbed in the stomach and the small intestine (DES, 2013), where its absorption, distribution, detoxification and elimination are well controlled (Kumar *et al.,* 2015). Copper homeostasis maintains of copper distribution and prevents causing any negative effects to cellular defense system (Quamar *et al.,* 2019). However, both augmentation and deficiency of copper concentration may cause physiological disorders (Chambers *et al.,* 2010). Thus, increases in copper concentration in body have been reported to be associated with many pathological conditions (Parmar *et al.,* 2002; Ozcelik and Uzun, 2009) as anemia by the red blood destruction (DES, 2013), abnormal lipid profile (Burkhead and Lutsenko, 2013) and lower triglycerides concentrations (Wuolikainen *et al.,* 2014). Furthermore, high copper level provokes cell injury (Saravu *et al.,* 2007) by oxidizing cell membranes (James *et al.,* 1999; Saravu *et al.,* 2007), mitochondrial dysfunction and lowering antioxidant enzymes, leading to oxidative stress damage (Tiwari *et al.,* 2018).

Through the years, interest of using plant compounds has been growing faster in worldwide due to their benefits on health (Nandi and Ghosh, 2016). Hawthorn, *Crataegus monogyna,* is one of very common shrub plant used in medicinal treatments (Fong and Bauman, 2002), which considered a relatively safe herb and without serious adverse effects (Zapfe, 2001). The plant is well distributed in the Mediterranean region. *C. monogyna* is rich in proanthocyanidins and flavonoids (Bahorun *et al.,* 1996), which are superoxide anion (Keser *et al.,* 2014), hydroxyl radical, hydrogen peroxides scavengers and lipid peroxidase reducer (Bahorun *et al.,* 1994 ; Rice-Evans, 2004), which make it a powerful antioxidant (Yao *et al.,* 2008). Interestingly, flavonoids of Hawthorn have the ability to inhibit copper intake (Kuo *et al.,* 1998).

The aim of this study is to investigate the ability of the common *C.monogyna aqueous extract* of both fruits and leaves in protecting blood

biomarkers and lipid profile of Wistar rat intoxicated with copper sulfate.

MATERIALS AND METHODS

Plant and preparation

Crataegus monogyna is grown spontaneously along the Algeria northern zone, exceeding 3 meters in length, and characterized by green leaves, white flowers and red fruits; the latter reaches maturity in mid-autumn. Fruits and leaves were harvested freshly in November from Annaba area, northeastern Algeria. 1.5g/kg bwe of fruits (F) and leaves (L) were weighted daily, crushed in an appropriate volume of distilled water (where each rat takes 1ml of the obtained extract) and were kept overnight at room temperature. The two homogenates were filtered in the morning for obtaining the of F and L *aqueous extract*. Copper sulfate powder was dissolved daily directly before carrying out the tests in distilled water. The mixture of copper + F and copper + L where prepared daily using the same doses.

Experimental design

Wistar rats were purchased from the Pasteur institute, Algiers (Algeria) weighing 196±8 g, that received tap water and standard diet *ad libitum*. Thirty-six males were divided equally into 6 groups; the control (C) having a standard diet, the copper (Cu: 100 mg $CuSO_4.5H_2O$/Kg bw), the fruits (F:1.5 g fruits/kg bw), the leaves (L: 1.5 g leaves/ kg bw), the Cu+ F (100 mg $CuSO_4.5H_2O$ /Kg bw + 1.5 g fruits/kg bw) and the Cu+ L (100 mg $CuSO_4.5H_2O$ /Kg bw + 1.5 g leaves/kg bw) group. Rats were sacrificed by decapitation after 30 consecutive days of copper oral administration, fruits and leaves solutions. Blood was collected in heparinized and EDTA test tubes, in which heparinized tubes were centrifuged at 3000 rpm for 10 minutes, and then the plasma was stored at -20°C till further analysis. Animals' experiments were authorized by the Ethical Committee of Animal Sciences at the University at the Badji Mokhtar university of Annaba (Algeria).

Erythrocytes G6PD assay

Glucose-6-phosphate deshydrogenase (G6PD) dosage was measured using Mindray BS-380 apparatus, according to BIOLABO REAGENT (U.V Kineticmethod) kit and the reaction scheme (Beutler *et al.,* 1977). The rate of increase in NADPH concentration measured at 340 nm is proportional to the G6PD activity of the specimen.

Complete blood count

The complete blood count was realized by using the blood counter Abacus 4.

Triglycerides assay

Triglyceride has been assayed using the enzymatic colorimetric method; according to the technical user manual of the Spinreact Kit (Spain). The triglycerides incubated with lipoprotein lipase (LPL) release the glycerol and free fatty acids. Glycerol is converted to glycerol-3-phosphat (G3P) and adenosine-5-diphosphate (ADP), by the glycerol kinase and adenosine phosphate (ATP). The G3P is then converted by the glycerol phosphate dehydrogenase (GPO) in active ingredient to dihydroxyacetone phosphate (DAP) and hydrogen peroxidase (H_2O_2). The latter reacts with 4-aminophenazone (4-AP) and p-chlorophenol in the presence of peroxidase (POD) to give a red color (Bucolo and David 1973).

HDL Cholesterol assay

The dosage of high density lipoproteins HDL has been carried out by the enzymatic method (Spinreact Kit, Spain).The very low density (VLDL) and low density (LDL) lipoproteins were precipitated by phosphotungstate in the presence of magnesium ions. After centrifugation the supernatant contains high density lipoproteins (HDL). The HDL cholesterol fraction was determined using the total cholesterol enzymatic reagent (Naito, 1984; Grove, 1979).

LDL cholesterol assay

The dosage of low density lipoproteins (LDL) was assayed according to technical guide of Spinreact Kit, Spain. Direct determination of serum LDL (low-density lipoprotein cholesterol) levels was carried out without the need for any sample pre-treatment or centrifugation of the sample (Friedewald *et al*., 1972).

Cholesterol assay

The assay of high density lipoproteins (HDL) was realized by the enzymatic method according

to the technical data sheet of the Spinreact Kit, Spain (Naito, 1984).

RESULTS AND DISCUSSION

Hematological markers are presented in table 1 showed a significant decrease in Cu group in G6PD, RBC, HGB and HCT levels, contrary Cu has augmented the WBC, PLT and MCV levels compared to the control. No change in the group treated with Cu F, while Cu L group showed a significant increase in G6PD, RBC, HCT and MCV levels, and a significant decrease in WBC level compared to the control.

Table 2 represents the rat's lipid profile exposed to copper for one month. Our Results showed significant decreases in triglyceride and cholesterol, while HDL and LDL levels kept the same levels compared to the control. Cu F showed an augmentation in triglyceride, cholesterol and decreased the HDL and LDL levels compared to the Cu group. In the group treated with Cu L, triglyceride, cholesterol and HDL levels augmented significantly, while no change was observed in LDL level compared to the Cu group.

In this research, copper sulfate administrated to rat for one month decreased significantly the G6PD, RBC, HGB and HCT levels, while it increased WBC, PLT and MCV. Recently, high copper level decreased the RBC counts and HGB concentration in rats (Akomolafe *et al.,* 2014) as a result of erythrocytes hemolysis induced by the free copper ions. The low activity of G6PD in rats of the copper group might be related to the inhibition of the enzyme by copper ions, an enzyme responsible of the red cells protection from oxidative stress by maintaining the GSH level through NADPH generation (Joshi *et al.,* 2002). Moreover, toxic copper was reported to induce hemolysis, leading to red blood dysfunction (Savaru *et al.,* 2007), and disturbs the erythropoiesis by affecting iron metabolism in the intestinal tracts, where copper and iron are antagonists (Pmila *et al.,* 1991). The observed iron deficiency during high copper level has led to anemia (Eck and Wilson, 1989) and methaemoglobinaemia (Oldenquist and Salem, 1999; Ahasan *et al.,* 1994), which confirm that copper is involved in the erythropoiesis process (Samanta *et al.,* 2011). On the other hand, the MCV

Table 1: Mean ± SD of some hematological markers in the different groups after treatments by copper sulphate and *C. monogyna* for one month.

	Control	Cu	F	L	Cu F	Cu L
G6PD (mUI/10^9)	121.4 ± 0.9^{b}	83.3 ± 0.6^{d}	124.1 ± 0.8^{a}	121.1 ± 2^{b}	121 ± 0.6^{b}	101 ± 0.4^{c}
WBC (10^3/mm)	7.49 ± 0.35^{c}	12.04 ± 0.1^{a}	7.95 ± 0.8^{c}	7.87 ± 0.7^{c}	8.01 ± 0.9^{c}	9.87 ± 0.4^{b}
RBC (10^6/mm^3)	10.36 ± 0.3^{a}	8.22 ± 0.2^{e}	9.86 ± 0.4^{bc}	9.47 ± 0.2^{cd}	10.05 ± 0.03^{ab}	9.017 ± 0.06^{d}
PLT (10^3/mm^3)	317 ± 1.7^{c}	899 ± 2.4^{a}	316 ± 20.9^{c}	307 ± 8.1^{c}	305 ± 5.8^{c}	435 ± 30.4^{b}
HGB(g/L)	151.8 ± 5.04^{a}	133.8 ± 3.4^{c}	152 ± 3.6^{a}	152 ± 2.1^{a}	152 ± 0.8^{a}	144.6 ± 0.8^{b}
HCT (%)	51.04 ± 0.8^{b}	40.7 ± 0.7^{d}	52.4 ± 0.8^{a}	50.7 ± 0.7^{b}	50.4 ± 0.5^{b}	47.3 ± 0.8^{c}
MCV (fl)	51.6 ± 0.5^{a}	39.3 ± 0.8^{c}	51.1 ± 0.7^{a}	51 ± 0.6^{a}	51 ± 0.6^{a}	47.8 ± 0.7^{b}

Means that do not share the same letter are significantly different ($p<0.05$), according to one-way ANOVA, followed by Tukey test. G6PD: 6-phosphate; WBC: white blood cells; RBC: red blood cells; PLT: platelets, HGB: hemoglobin; HCT: hematocrits; MCV: mean corpuscular volume.

Table 2: Mean ± SD of Biochemical markers in the different groups after treatments by copper sulphate and *C. monogyna* for one month.

	Control	Cu	F	L	Cu F	Cu L
TRIG (g/l)	0.88 ± 0.07^{a}	0.13 ± 0.03^{d}	0.56 ± 0.02^{c}	0.63 ± 0.03^{b}	0.55 ± 0.03^{c}	0.55 ± 0.03^{c}
CHOL (g/l)	0.68 ± 0.007^{a}	0.21 ± 0.01^{c}	0.46 ± 0.01^{c}	0.53 ± 0.02^{b}	0.54 ± 0.02^{b}	0.55 ± 0.01^{b}
HDL (g/l)	0.31 ± 0.01^{e}	0.31 ± 0.01^{e}	0.56 ± 0.008^{a}	0.45 ± 0.007^{b}	0.37 ± 0.01^{c}	0.33 ± 0.008^{d}
LDL (g/l)	0.18 ± 0.008^{a}	0.17 ± 0.01^{ab}	0.10 ± 0.005^{c}	0.09 ± 0.001^{c}	0.11 ± 0.01^{c}	0.15 ± 0.01^{b}

Means that do not share the same letter are significantly different ($p<0.05$), according to one-way ANOVA, followed by Tukey test. TRIG: triglycerides; CHOL: cholesterol, HDL: high density lipoproteins; LDL: low density lipoproteins.

and HCT level have increased significantly when rats exposed to copper, without affecting RBC count and HBG concentration (Akomolafe *et al.,* 2016). Liver injury by the copper toxicos is may lead to coagulation cascade (Nelson, 2002); this perhaps explains the observed rise in PLT levels in our finding, which was not the case in the study of Ganong, (2009) who reported that high copper charge had decreased the PLT level by inhibiting the thrombopoietin production. Copper may cause inflammatory reactions to some organs such as liver, heart and kidneys, which may explain the increase of WBC as the macrophages that are sensitive to heavy metals toxicity (Witeska and Wakulska, 2007).

The combined treatment of copper and hawthorn fruits extract in this study showed an increase in G6PD activity and HCT levels, without affecting the other parameters. Thus, *C. monogyna* seems to play an important role in free radicals scavenging induced by copper sulphate, as the study of Bernatonienë *et al.* (2008), who indicated that aqueous and ethanolic extracts have the capacity in protecting cells from oxidative stress. Moreover, hawthorn was reported to be rich in polyphenols (Liu *et al.,* 2019), that have protective activity for hematological markers against lead toxicity (Aksu *et al*., 2012). Also, the active compounds in *C. monogyna* seem to have the ability to enhance the antioxidant system by rising G6PD activity to protect red blood cells against stress injuries. This enzyme is the main supplier of protons through the coenzyme NADP to generate reduced glutathione.

The remarkable triglycerides and cholesterol concentrations decrease in rats having toxic copper dose after thirty days consecutive exposure were in conformity with the studies of Mondal *et al.,* (2007) and Babaknejad *et al.,* 2015). The maintaining level of LDL and HDL in this investigation was probably linked to the HDL synthesis from LDL via the modulation of HMG-CoA reductase activity by copper (Mondal *et al.,* 2007). Contrary, copper administration to cows (40mg/kg) had led to a cholesterol concentration increase (Engle *et al.,* 2001) and cholesterol and LDL in rats as results of the oxidative stress (Galhardi *et al.,* 2004).

The *C. monogyna* administration in both L and F groups has reduced the triglycerides levels, cholesterol, and LDL, while it raised the HDL production. In fact hawthorn given to rats at 2% of the diet was demonstrated to have a hypocholesterolemic and vasoprotective activities (Kwok *et al.,* 2010). Researchers found that the alcoholic extract of the *C. monogyna* berries lowered significantly the cholesterol, triglycerides and the LDL levels (Kausar *et al.,* 2011). Furthermore, *C. monogyna* could increase the receptors capacity to bind to LDL and therefore prevent the cholesterol augmentation (Kausar *et al.,* 2011) and enhancing the cholesterol elimination to bile (Rajendran *et al.,* 1996). Hawthorn was also been found to decrease the serum levels of cholesterol, LDL-cholesterol, and triglycerides in hypercholesterolemic and atherosclerotic animals (Chang *et al.,* 2002). Also studies showed that hawthorn may lower the body weight as our results indicated, and it used to treat obesity and weight control (Kausar *et al.,* 2012).

In the combined group Cu L and Cu F, the HDL level increased significantly, which means that the hawthorn has a beneficial effect, explained by the presence of catalytic metal ions, that increase the long and short chain cholesterol ester and phospholipids (Abuja and Albertini 2001). While LDL decreased significantly particularly HDL with high copper concentration perhaps by accelerating the LDL oxidation (Raveh *et al.,* 2001).

CONCLUSION

The copper induced rat toxicity during thirty days has disturbed most blood parameters and lipid profile, while the co-administration of *C. monogyna* leaves and fruits extracts has led to a mitigating effect by normalizing many blood biomarkers.

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REFERENCES :

Abbas, B. F., Al-Jubori, W. M. K., Abdullah, A. M., Sha'aban, H. K and Mohammed, M. T. 2018. Environmental pollution with the heavy metal compound. *Research Journal of Pharmacy and Technology*, **11**(9): 4035-4041.

Abuja, P. M. and Albertini, R. 2001.Methods for monitoring oxidative stress, lipid peroxidation and oxidation resistance of lipoproteins. *Clinicachimicaacta*, **306**(1-2): 1-17.

Ahasan, H. N., Chowdhury, M. J., Azhar, M. A. and Rafiqueuddin, A. K. M. 1994. Copper sulphatepoisoning. *Tropical doctor*, **24**(2): 52-53.

Akomolafe, R. O., Olukiran, O. S., Imafidon, C. E., Ayannuga, O. A., Oyekunle, J. A., Akanji, B. O. and Oladele, A. A. 2014. A study of two weeks administration of copper sulphate on markers of renal function and feeding pattern of Wistarrats. *African Journal of Biochemistry Research*, **8**(9): 158-165.

Akomolafe, R. O., Olukiran, O. S., Imafidon, C. E., Ayannuga, O. A., Oyekunle, J. A., Akanji, B. O. and Oladele, A. A. 2016. Effects of chronic copper sulphate administration on feeding pattern and markers of renal and liver functions of Wistarrats. *Physiology*, **26**.1(89):13-19

Aksu, D. S., Didin, M. and Kayikci, F. 2012. The protective role of polyphenols on blood cells in rats exposed to lead. *RevistaRomânã de Medicinã de Laborator*, **20**(3/4): 47-57.

Babaknejad, N., Moshtaghie, A.A., Shahanipour, K. and Bahrami, S. 2015. Effect of zinc, magnesium and copper on lipid profile as a risk factor of cardiovascular disease in male Wistarrats. *Journal of Preventive Cardiology*, **4**(3) 692-697.

Bahorun, T., Gressier, B., Trotin, F., Brunet, C., Dine, T., Luyckx, M., Vasseur, J., Cazin, M., Cazin, J.C. and Pinkas, M. 1996. Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical preparations. *Arzneimittel-forschung*, **46**(11): 1086-1089.

Bahorun, T., Trotin, F., Pommery, J., Vasseur, J. and Pinkas, M. 1994. Antioxidant activities of *Crataegus monogyna* extracts. *Planta medica*, **60**(04): 323-328.

Bernatonienë, J., Masteikova, R., Majienë, D., Savickas, A., Këvelaitis, E., Bernatonienë, R., Dvoráèková, K., Civinskienë, G., Lekas, R., Vitkevièius, K. and Peèiûra, R. 2008. Free radical-scavenging activities of *Crataegus monogyna* extracts. *Medicina*, **44**(9): 706-12.

Beutler, E., Blume, K.G., Kaplan, J.C., Löhr, G.W., Ramot, B. and Valentine, W.N. 1977. International comittee for standardisation in haematology: recommended methods for red cell enzyme analysis. *British Journal of Haematology*, **35**: 331-340

Bucolo, G. and David, H. 1973. Quantitative determination of serum triglycerides by the use of enzymes. *Clinical chemistry*, **19**(5): 476-482.

Burkhead, J. L. and Lutsenko, S. 2013. The role of copper as a modifier of lipid metabolism.In Lipid metabolism. IntechOpen.

Chambers, A., Krewski, D., Birkett, N., Plunkett, L., Hertzberg, R., Danzeisen, R., Jonesk, P., Keenl, C. L., Meekm, B., Schoenyn, R. and Slob, W. 2010. An exposure-response curve for copper excess and deficiency. *Journal of Toxicology and Environmental Health*, Part B, **13**(7-8): 546-578. .

Chang, Q., Zuo, Z., Harrison, F. and Chow, M. S. S. 2002. Hawthorn. *The Journal of Clinical Pharmacology*, **42**(6), 605-612.

DES, N. 2013. Copper: Health information summary. Concord, NH, USA: *New Hampshire Department of Environmental Services*.

Eck, P. C. and Wilson, L. 1989. Copper toxicity. Eck Institute of Applied Nutrition and Bioenergetics Ltd, 1-16.

Engle, T. E., Fellner, V. and Spears, J. W. 2001. Copper status, serum cholesterol, and milk fatty acid profile in Holstein cows fed varying concentrations of copper. *Journal of Dairy Science*, **84**(10): 2308-2313.

Fong, H.H. and Bauman, J. L. 2002. Hawthorn. *Journal of Cardiovascular Nursing*, **16**(4), 1-8.

Friedewald, William, T., Robert, I. Levy, and Donald, S. Fredrickson. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, **18 (6)**:499-502.

Galhardi, C. M., Diniz, Y. S., Faine, L. A., Rodrigues, H. G., Burneiko, R. C., Ribas, B. O. and Novelli, E. L. 2004. Toxicity of copper

intake: lipid profile, oxidative stress and susceptibility to renal dysfunction. *Food and chemical toxicology*, **42**(12): 2053-2060.

Ganong, W.F. 2009. *Review of Medical Physiology*, 23rd ed, Mc Grow Hill, New York , pp 65

Grove, T.H. 1979. Effect of reagent pH on Determination of HDL Cholesterol by precipitation with Sodium Phosphotungstate-magnesium. *Clinical Chemistry*, **25**:560.

Jalali, A. S., Hasanzadeh, S. and Malekinejad, H. 2012. *Crataegus monogyna aqueous extract* ameliorates cyclophosphamide-induced toxicity in rat testis: stereological evidences. *Acta MedicaIranica*, **50**(1):1-8.

James, L. P., Stowe, C. D. and Argao, E. R. I. C. 1999.Gastric injury following copper sulfate ingestion. *Pediatric emergency care*, **15**(6): 429-431.

Joshi, P. K., Bose, M. and Harish, D. 2002. Haematological changes in the blood of Clariasbatrachus exposed to mercuric chloride. *Journal of Ecotoxicology & Environmental Monitoring*, **12**(2): 119-122.

Kausar, S., Zaheer, Z. and Saqib, M. 2012. Role of Crataegus (Hawthorn) Extract on Obesity in Hyperlipidemic Albino Rats. *Pakistan Journal of Medical and Health Sciences*, **6**(1):100-103.

Kausar, S., Zaheer, Z., Saqib, M. and Zia, B. U. S. H. R. A. 2011. The effect of Crataegus (Hawthorn) extract alone and in combination with simvastatin on serum lipid profile in hyperlipidemic albino rats. *Biomedica*, **27**(2): 140-7.

Keser, S., Celik, S., Turkoglu, S., Yilmaz, Ö and Turkoglu, I. 2014. The investigation of some bioactive compounds and antioxidant properties of hawthorn (*Crataegus monogyna* subsp. monogyna Jacq). *Journal of intercultural ethnopharmacology*, **3**(2): 51-55.

Kumar, A., Gupta, T., Berzsenyi, S. and Giangrande, A. 2015. N-cadherin negatively regulates collective Drosophila glial migration through actin cytoskeleton remodeling. *Journal of Cells Sciences*, **128**(5): 900-912.

Kuo, S. M., Leavitt, P. S. and Lin, C. P. 1998. Dietary flavonoids interact with trace metals and affect metallothionein level in human intestinal cells. *Biological Trace Element Research*, **62**(3): 135-153.

Kwok, C. Y., Wong, C. N. Y., Yau, M. Y. C., Yu, P. H. F., Au, A. L. S., Poon, C. C. W., Sai-Wang. S., Tsz-Y.L., Yiu-Wa, K and Chan, S. W. 2010. Consumption of dried fruit of *Crataegus pinnatifida* (hawthorn) suppresses high-cholesterol diet-induced hypercholesterolemia in rats. *Journal of Functional Foods*, **2**(3): 179-186.

Liu, H., Liu, J., Lv, Z., Yang, W., Zhang, C., Chen, D. and Jiao, Z. 2019. Effect of dehydration techniques on bioactive compounds in hawthorn slices and their correlations with antioxidant properties. *Journal of food science and technology*, **56**(5): 2446-2457.

Mondal, M. K., Das, T. K., Biswas, P., Samanta, C. C. and Bairagi, B. 2007. Influence of dietary inorganic and organic copper salt and level of soybean oil on plasma lipids, metabolites and mineral balance of broiler chickens. *Animal feed science and technology*, **139**(3-4): 212-233.

Naito, H.K.1984. High-density lipoprotein (HDL) cholesterol.Kaplan A *et al. Clinical Chemistry*.The C.V. Mosby Co. St Louis. Toronto. Princeton; 1207-1213 and437.

Nandi, P. and Ghosh, S. N. 2016. Effect of medicinal plants as intercrop on plant and soil of Mosambi sweet orange gown in laterite oil. *International Journal of Minor Fruits, Medicinal and Aromatic Plants*, **2**(2): 11-13.

Nelson, L.S. 2002. Copper. In: Goldfrank LR, Flomenbaum NE, Lewin NA, editors. Goldfrankstoxicologic emergencies7th ed, McGraw-Hill: New York, 1262-71.

Oldenquist, G. and Salem, M. 1999. Parenteral copper sulfate poisoning causing acute renal failure. *Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association-European Renal Association*, **14**(2): 441-443.

Ozcelik, D. and Uzun, H. 2009. Copper intoxication; antioxidant defenses and oxidative damage in rat brain. *Biological trace element research*, **127**(1), 45-52.

Parmar, P., Limson, J., Nyokong, T. and Daya, S. 2002. Melatonin protects against copper

mediated free radical damage. *Journal of pineal research*, **32**(4): 237-242.

Pmila, D., Subbaiyan, P. S. and Ramaswamy, M. 1991. Toxic effect of chromium and cobalt on sarotherodonmossambicus. *Indian Journal of Environmental Health,* **33***:* 218-224.

Quamar, S., Kumar, J., Mishra, A. and Flora, S. J. S. 2019. Oxidative stress and neuro-behavioural changes in rats following copper exposure and their response to MiADMSA and d-penicillamine. *Toxicology Research and Application*, **3**:https://doi.org/10.1177/2397847319844782

Rajendran, S., Deepalakshmi, P. D., Parasakthy, K., Devaraj, H. and Devaraj, S. N. 1996. Effect of tincture of Crataegus on the LDL-receptor activity of hepatic plasma membrane of rats fed an atherogenic diet. *Atherosclerosis*, **123**(1-2): 235-241.

Raveh, O., Pinchuk, I., Fainaru, M. and Lichtenberg, D. 2001. Kinetics of lipid peroxidation in mixtures of HDL and LDL, mutual effects. *Free Radical Biology and Medicine*, **31**(11): 1486-1497.

Rice-Evans, C. 2004. Flavonoids and isoflavones: absorption, metabolism, and bioactivity. *Free Radical Biology and Medicine*, **7**(36): 827-828.

Samanta, B., Ghosh, P. R., Biswas, A. and Das, S. K. 2011.The effects of copper supplementation on the performance and hematological parameters of broiler chickens. *Asian-Australasian Journal of Animal Sciences*, **24**(7): 1001-1006.

Saravu, K., Jose, J., Bhat, M. N., Jimmy, B. and Shastry, B. A. 2007. Acute ingestion of copper sulphate: A review on its clinical manifestations and management. *Indian Journal of Critical Care Medicine*, **11**(2): 74-80.

Tiwari, M. K., Leinisch, F., Sahin, C., Møller, I. M., Otzen, D. E., Davies, M. J. and Bjerrum, M. J. 2018. Early events in copper-ion catalyzed oxidation of á-synuclein. *Free Radical Biology and Medicine*, **121**, 38-50.

Witeska, M. and Wakulska, M. 2007.The effects of heavy metals on common carp white blood cells in vitro. *Alternatives to Laboratory Animals*, **35**(1): 87-92.

Wuolikainen, A., Acimovic, J., Lövgren-Sandblom, A., Parini, P., Andersen, P. M and Björkhem, I. 2014.Cholesterol, oxysterol, triglyceride, and coenzyme Q homeostasis in ALS. Evidence against the hypothesis that elevated 27-hydroxycholesterol is a pathogenic factor. *PloS one*, **9**(11), e113619.

Yao, M., Ritchie, H. E. and Brown-Woodman, P. D. 2008. A reproductive screening test of hawthorn. *Journal of ethnopharmacology*, **118**(1): 127-132. (H32).

Zapfe, G. J. 2001. Clinical efficacy of Crataegus extract WS® 1442 in congestive heart failure NYHA class II. *Phytomedicine*, **8**(4): 262-266.

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Phenolic compound profiles and antioxidant activity of *Ruta chalepensis L.* leaves, a spontaneous medicinal herb: influence of harvest zone (Western Algeria)

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ABSTRACT

Natural plant extracts contain a variety of phenolic compounds, to which various biological activities are attributed. Ruta chalepensis, known as "Fidjel", is widespread in the Algerian territory, which was selected in order to estimate its content of secondary metabolites (phenolic compounds, flavonoids, tannins), and its antioxidant activity (DPPH, FRAP). For this purpose, two methanolic extracts were prepared from the leaves of this plant, harvested from two different areas: Tessala mountains (Sidi-Bel-Abbes town) and Taougrite mountains (Chlef town), which crude extract yields are around of 12.4% and 20.1% respectively. The quantitative estimation of the different secondary metabolites showed that the methanolic extract of Tessala mountains (MER_{TES}) is the richest in polyphenols (10.65±0.67 mg GAE/g), flavonoids (31.16 ± 0.55 mg CE/g), hydrolyzable tannins (0.78 ± 0.02 mg CE / g), and in condensed tannins (2.75 ± 0.10 mg CE/g). However, an antioxidant activity evaluation of the two extracts showed that, both fractions are active with a maximum IC_{50} of 68.41±9.98 µg/ml in (MER_{TES}). On the other hand, the extract from that of the Taougrite Mountains (MER_{TAO}) shows a stronger reducing activity than (MER_{TES}); the difference remains not significant. In conclusion, the plant harvesting area, and the bioclimatic conditions might influence the Rutaceae leaf extracts content and quality of the same family.

Keywords: DPPH, FRAP, methanolic extract, *Ruta chalepensis*, secondary metabolites, yield.

INTRODUCTION

Since the beginning of humanity, people have been depending on plants to provide their vital necessities, thus the important role of plants as a good source of medicines (Sabitha Rani *et al.*, 2019). In modern medical aspects, various plants or plant-based materials have attracted a great focus on the potential drug candidate development/ extraction, particularly for the non-communicable diseases treatment such as diabetes mellitus and cancer, etc. (Bhowmik, 2019). The active principles of medicinal plants are often linked to the productions of secondary metabolites, which are widely used in therapy, such as preventive anti-inflammatory, antimicrobial, antiseptic, diuretic agents, essentially antioxidant agents which defend against oxidative stress (Bourgaud *et al.*, 2001; Kar, 2007). Phenolic compounds, essential oils and other secondary metabolites represent high value molecules, used in the pharmaceutical, cosmetic and food industries. The antioxidant activities of these products have been reported in numerous studies around the world (Bouzouita *et al.*, 2008). The *Ruta chalepensis* plant, belonging to the *Rutaceae* family is very rich in secondary metabolites, which explains its biological activities: antifungal, antioxidant and anti-inflammatory (Gonzalez-Trujano *et al.*, 2006; Raghav *et al.*,2006; Al-Said *et al.*,1990). This specie is spontaneous, widely distributed in North Africa, particularly in Algeria; where it is known as *Fidjel*.

The objective of our study is to evaluate *in vitro* the antioxidant activity, as well as, the content of total phenolic compounds, flavonoids,

hydrolyzable and condensed tannins of *Ruta Chalepensis methanolic extracts*, collected before the flowering period. Sampling was undertaken in two different western Algeria areas, (Tessala mountains in Sidi-Bel-Abbes region and Taougrite mountains in Chlef region) characterized by different bioclimatic conditions with the purpose to assess whether the different climatic conditions may influence the bioactive compound content.

MATERIALS AND METHODS

Plant material

Ruta Chalpensis was harvested in February 2018 in two different, areas: Tessala mount, which is located at 15 km northwest of the Sidi-Bel-Abbes city and the Taougrite mountain of the Chlef city. Bioclimatic data for the two regions are listed in (Table 1).

The leaves were washed in running water, and then dried at room temperature and away from light. The samples were then crushed and sieved to obtain a homogeneous granular structure, and stored in glass vials for further analysis (Fig. 1).

Preparation of *methanolic extracts*

The powdered *Ruta Chalepensis* leaves (10 g) were extracted with 100 ml of 80% methanol, under agitation for 24 hours at room temperature (Majheniè *et al.*, 2007). The mixture was filtered and then concentrated using a rotavapor (Heidolph instruments), to obtain two *extract*: MER_{TES} = *methanolic extract* of *Ruta* from Tessala mount; MER_{TAO} = *methanolic extract* of *Ruta* from Taougrite mount.

Determination of secondary metabolites

Total phenols

The total phenol content of the *extracts* was determined by the Folin-Ciocalteu method (Qusti *et al.*, 2010). 200 µl of the *extract* was mixed with 1 ml of freshly prepared Folin-Ciocalteu reagent (ten times diluted), and 0.8 ml of 7.5% sodium carbonate (Na_2CO_3) has been added. The mixture was incubated at room temperature for 30 minutes, and the reading was taken against a blank using a spectrophotometer at 765 nm. Phenol concentration in each sample was calculated against a calibration curve with gallic acid at different concentrations. The results are expressed as milligrams gallic acid equivalent per gram of dry matter (mg GAE/g d.w).

Flavonoids

The flavonoid content of the *extracts* was determined using the aluminum trichloride colorimetric method (Kim *et al.*, 2003). An amount of 500 µl of the *extract* was mixed with 1.5 ml of distilled water, and subsequently with 0.3 ml of a 5% sodium nitrite solution $NaNO_2$. After five minutes, 3 ml of a 10% $AlCl_3$ solution was added. After 6 min, 1 ml of 4% NaOH was added. After five minutes the whole mixture was stirred with a vortex. The absorbance was measured at 510 nm. Quercetin was used as standard for the calibration curve. Total flavonoids contents are expressed as, mg quercetin equivalent/g of dry matter (mg CE/g d.w).

Condensed tannins

This determination rate method of the condensed tannins is based on the condensation of polyphenolic compounds with vanillin in an acidic medium, which will give a brown compound. For the determination of condensed tannins, 0.1 ml-0.5 ml *extracts* were placed in tubes and then 3 ml of 4% (w/v) vanillin in methanol are added. After vigorous stirring 1.5 ml of concentrated HCl was immediately added and stirred again. Absorbance was measured at 500 nm after 20 min of incubation (Julkunen-Titto,1985).The calibration curve was prepared under the same conditions, using catechin as standard and the results are expressed as mg catechin equivalent/g dry matter (mg CE/g).

We have adopted the vanillin method with HCl. Which depends on the vanillin reaction with the terminal flavonoid group of condensed tannins, with the red complexes formation, this is explained by the characteristic of tannins to transform into red colored anthocyanidols by the reaction with vanillin. The content of condensed tannins was determined by the vanillin method described by (Julkunen-Titto,1985). A volume of 50 ìl of each *extract* was added to 1500 ìl of the 4% vanillin/ methanol solution and mixed vigorously. Then, 750 ìl volume of concentrated hydrochloric acid (HCl) was added. The obtained mixture was allowed to react at room temperature for 20 min. The absorbance was measured at 550 nm against a control. Various concentrations between 0 and 1000 µg/ml prepared from a catechin stock solution, will be used to trace the calibration curve.

Hydrolyzable tannins

The hydrolyzable tannins determination was performed by the Mole and Watrman method (1987), based on a reaction with ferric chloride. The *tannic extract* mixture with the ferric chloride reagent produces a blue-black coloration in the presence of gallic tannins; and a green-brown coloration in the presence of catechic tannins, from which the (Fe3+) ions are formed. To accomplish this, 1 ml of the *extract* was added to 3.5 ml of a 0.01M Fe $C1_3$ solution in 0.001M HCl (V/V). The mixture was vigorously mixed, and the optical density was read at 660 nm with a spectrophotometer. The hydrolyzable tannins content in the *extracts* was calculated from a calibration curve, carried out with gallic acid, under the same experimental conditions as the tested samples

A 0.01 M Fe Cl_3 solution was mixed with a 0.001M (v/v) HCl solution. 3.5 ml of this solution was added to 1ml of *extract*. After 15 seconds, the absorbance was measured at 660 nm.

The hydrolyzable tannins are expressed by the following formula:

H T (%) = (Abs x M x V) / E mole x W

With: HT: hydrolysable tannins, Abs: absorbance, E mole: 2169 of gallic acid (constant expressed in mole), M: mass = 300, V: volume of the used *extract*, W: weight of the sample. The results are expressed as milligram gallic acid equivalent per gram of dry *extract* (mg GAE/g DE).

Antioxidant activity

DPPH Test

In the presence of free radical scavengers, the purple-colored DPPH (2,2-diphenyl-1-picrylhydrazyl) is reduced to yellow-colored 2,2-diphenyl-1-picrylhydrazine (Maataoui *et al.*, 2006 and Molyneux, 2004).

The free radical scavenging activity of plant *extracts* was determined according to (Benhammou *et al.,* 2009). A volume of 50 ìl of different concentrations of each *extract* was added to 1.95 ml of the freshly prepared DPPH methanolic solution (25 mg/l). The negative control, were prepared in parallel, by mixing 50 ìl methanol with 1.95 ml of a methanolic DPPH solution, at the same concentration. After incubation in the dark for 30 minutes at room temperature, the absorbances of each sample and negative control were read at 515 nm using a spectrophotometer. The reactions were repeated three times for each dilution, and then the value mean of the percentage inhibition of scavenging activity for DPPH was taken.

Calculation of inhibition percentages of DPPH

We calculate the inhibition percentages by the following formula :

I % = ((Ca - Ta) / Ca) × 100. With: Ca: the control absorbance; Ta: the test absorbance performed.

All tests were performed in triplicate. The *methanolic extracts* kinetics reactions and ascorbic acid with DPPH were recorded at each examined concentration. The concentrations of the different *extracts* and ascorbic acid, as a function of the percentages of inhibited DPPH, were graphed at the end of the reactions to obtain the IC_{50} index.

IC_{50} or 50% inhibitory concentration is the test sample concentration required to reduce 50% of the DPPH radical. The IC_{50} was graphically calculated by the linear regressions of the graphical representations of the inhibition percentages as a function of different concentrations of the fractions tested.

FRAP Test (Ferric Iron Reducing Power)

The principle is based on the ferric iron reduction reaction (Fe^{3+}), present in the $K_3Fe(CN)_6$ complex to ferrous iron (Fe^{2+}) by an antioxidant, the reaction is revealed by the change of the yellow color of ferric iron (Fe^{3+}) to the blue-green color of ferrous iron (Fe^{2+}).The intensity of this coloration is measured by spectrophotometry at 700 nm (Chung *et al.,* 2002).

The protocol established by (Oyaizu *et al.,*1986) was adopted, which consists of taking 0.5 ml of each *extract*, at different concentrations and mixing them with 1.25 ml of a 0.2M phosphate buffer solution (pH = 6.6) and 1.25 ml of a 1% potassium ferricyanide solution $K_3Fe(CN)_6$. The mixture was incubated at 50°C, for 20 min, and then cooled at room temperature. 2.5ml of 10% trichloroacetic acid was added to stop the reaction, and then the tubes are centrifuged at 3000 rpm for 10 min. Then 1.25ml of the supernatant added to 1.25 ml of distilled water and 250 ml of 0.1% (Fe Cl_3) solution. The absorbances read spectrophotometrically at a wavelength of 700 nm. The positive control was

represented by an antioxidant standard solution; the ascorbic acid, the absorbance of which was measured under the same conditions as the samples. The iron (Fe^{3+}) reducing activity determination was performed in triplicate.

Statistical analysis

The results are expressed in the form of the mean and their standard (X ± ES). Statistical analysis of the data is conducted using Microsoft Excel version 2010 software. The statistical analysis of the different groups data; was carried out by the Student test "t"; this parametric statistical test is suitable for a comparative analysis between the means of the experimental samples, and that of the control group. In all cases, a p value <0.05 was considered significant.

RESULTS AND DISCUSSION

Extractions yield

The *Ruta chalepensis* leaves *crude extract* yields changed according to the stations (Table 2). The highest yields were measured in the plant leaves of the Taougrite mountains MER_{TAO} (20.1%) whereas in Tessala MER_{TES} were 12.4 %.

The difference in yield rate obtained was due before hand to the solvent used on the one hand and on the other hand, to the richness of *Ruta chalepensis* in methanol-soluble substances. Indeed, studies have indicated that a ratio of methanol 70% is generally used in the flavonoids extraction, phenol acids and their derivatives; and a wide range of biomolecules (Al-Farsi and Lee, 2008). The results of (Al-Said *et al.*,1990) on *R. chalepensis*, gave a *crude extract* yield of the whole aerial parts with 3.75%, this yield is clearly much lower than that obtained in our study, this may be due to characteristics of each species and the harvest region (soil, temperature). Thus, for *Ruta chalepensis*, the *crude methanolic extracts* yields found was higher than those reported by (Merghache *et al.,* 2009) (0.82%), (Hnatyszyn *et al.,* 1974) (0.9%), (Mejri *et al.,* 2010) (5.51%) and (Fakhfakh *et al.*, 2012) (2.32 to 1.25%).

Total phenols and flavonoids content

The levels of total phenols and flavonoids are reported in (Fig. 2). According to our results, we noticed that MER_{TES} was richer in flavonoids ($p < 0.05$) in comparison with MER_{TAO}. While, the difference in polyphenol content in the two *extracts* remains statistically insignificant ($p > 0.05$).

The difference in total phenols and flavonoids content may be due to the climatic conditions, which differ from region to another. The polyphenol content variability in plant species is probably due to, the *extracts* phenolic composition (Hayouni *et al.,* 2007), to genotypic factors, biotic (species, organ and physiological stage), abiotic (edaphic factors) (Ksouri *et al.,* 2008), the soil nature and the microclimate type (Atmani *et al.*, 2009), and also to the bioclimatic stages where these plants grow. On the other hand, Bentabet *et al*.(2007) confirmed that the polyphenolic content varies both qualitatively and quantitatively from plant to another, which can be attributed to several factors such as climatic and environmental factors, genetic patrimony, the harvesting period and the stage of plant development, the *extract* concentrations, the extraction used method, etc.

The polyphenol content of tested *extracts* was lower compared to the study realized by Bettaieb *et al*.(2012), who reported that the total phenol content in the *Ruta chalepensis* aerial part obtained from *methanolic extract* was around 13.7 mg GAE/g of dry matter, while Ghazghazia *et al.* (2013) have reported that, the *R. chalepensis* leaves polyphenol contents are 12.82 mg GAE/g of dry matter. In another study carried out by Shuib *et al.* (2015), the *Ruta angustifolia* species registered a content of 18.89 mg GAE/g.

Table 1: Climatic features of the study areas

Characters	Longitude	Latitude	Altitude	Climate
Tessala	00:76408°	35:26978°	1061 m	Semi-arid dry and cold
Taougrite	0°552 222 2	36°142 392 2	528 m	Mediterranean with hot summer

Table 2: Crude *extracts* yields from *Ruta chalepensis* leaves.

Samples	Yield (%)
MER_{TES}	12.4
MER_{TAO}	20.1

On the other hand, the tested plant flavonoid content remained higher compared to other researches performed by Khlifi *et al.* (2013) who reported the *Ruta chalepensis methanolic extract* aerial part flavonoid content to be around 12.78 ± 0.08mg EAG/g of dry matter. While Bettaieb *et al.* (2012) registered a content of 6.50 mg QE/g of *hydro-methanolic extrac*t (80:20) (V:V). This signifies that the flavonoid content varied according to the used extraction solvent.

Condensed and hydrolyzable tannins content

The condensed and hydrolyzable tannins quantification is shown in (Fig.3). Our results confirm that, the plant *extract* from the Tessala mountains is slightly rich in hydrolyzable ($p<0.05$) and condensed ($p<0.01$) tannins, compared to that from the Taougrite mountains. In general, the chemical families detected in our experimentation, confirmed the researches carried out on various origins species (Saudi Arabia, Turkey, Jordan, Algeria, India, Morocco and Oman) (El-Sayed *et al.,* 2000; Gunaydin and Savci, 2005; Shehadeh *et al.,* 2007; Haddouchi *et al.*,2013; Raaman *et al.,* 2014; Al-Brashdi *el al.,* 2016; Daoudi *et al.,* 2016).

Indeed, the presence of these secondary metabolites can explain various biological activities: protective against biotic and abiotic aggressions, antifungal, antioxidant, phytotoxic, abortive, anti-allergic, anti-tumoral, anticancer, neuroprotective, antispasmodic, cytotoxic, antibacterial, anti-inflammatory, antiviral and insecticides (Conti *et al.*,2013; Shuib *et al.,* 2015; Chaibeddra *et al.,* 2016; Daoudi *et al.*,2016).

Antioxidant activity

DPPH (2.2'-diphenyl-1-picrylhydrazyl)

The results shown in the figure 4, illustrate the percentages of the antiradical activity of the two *Ruta chalepensis L. extracts* shows that MER_{TES} exhibits the highest inhibition percentage of order 85.6% at maximum concentration (250µg/ml), followed by MER_{TAO} with a value of 84.35%.

Among the obtained different fractions, the MER_{TES} fraction represents the most active *extract*, its IC_{50} was 68.41 ± 9.98 µg /ml, followed by the MER_{TAO} fraction with an IC_{50} around 70.6 ±12.12 µg/ml. Compared to the standard antioxidant ascorbic acid, both fractions are more active. The difference between the IC_{50} of the tested *extracts* was not statistically significant (Fig.5).

Both *extracts* showed significant antioxidant capacity. According to Abou Zeid *et al.*(2014), phenolics such as flavonoids, phenolic acids, tannins and furocoumarins directly contribute to the plant antioxidant capacity. Turkmen *et al.* (2007) noted that polyphenols appear to be effective hydrogen donors to the DPPH radical, due to their chemical structure. Works carried out by Kang *et al.* (2003) suggested that, the polar molecules present in the plant *extracts* contribute to the antioxidant activity increase. This is generally due to the synergy between the different existing antioxidant compounds. Similarly, studies by Ouerghemm *et al.* (2016), which were performed on the *R. chalepensis* flowers *methanolic extract* from two different provenances (wild and cultivated), revealed that the antioxidant potential was quite high in the plant's aerial part with an IC_{50} in the range of 23.73ìg/ml for the wild flowers and 28.48ìg/ml for the cultivated *R. chalepensis* flowers. The study achieved by Ghazghazi *et al.* (2013) on the *Ruta chalepensis* antioxidant activity revealed a remarkable antioxidant capacity; IC_{50} of the leaves wich was estimated at 35ìg/ml. The antioxidant capacity seems to be influenced by the total polyphenols contents. The studies of Kacem *et al.* (2015) also showed that *R. chalepensis* possesses a fairly high antioxidant potential. Indeed, at a concentration of about 500 mg/ml, the *ethanolic extract* decolors the DPPH and scavenges the free radicals with an inhibition rate of about 84%.

Iron reducing (FRAP*)*

The reducing activity results clearly show that, MER_{TAO} exhibits the reduce power of Fe^{+3} ion more strongly in comparison with MRE_{TES}, the statistical analysis doesn't show a significant difference ($P> 0.05$) (Fig. 6). According to results obtained, the activity of our *extracts* is average, these results which synchronize with that of Djeridane *et al.* (2006), where they found that, the *Ruta sp. phenolic extract* activity was less important compared to the other ten plants studied at the same time, although the phenolic compounds content was important, this is generally due to the synergy between the different existing antioxidant compounds, which makes the activity not only concentration-dependent. The antioxidant activity depends on several factors, such

Fig. 1: *Ruta chalepensis* fresh plant (A) and after drying (B) (personal photo)

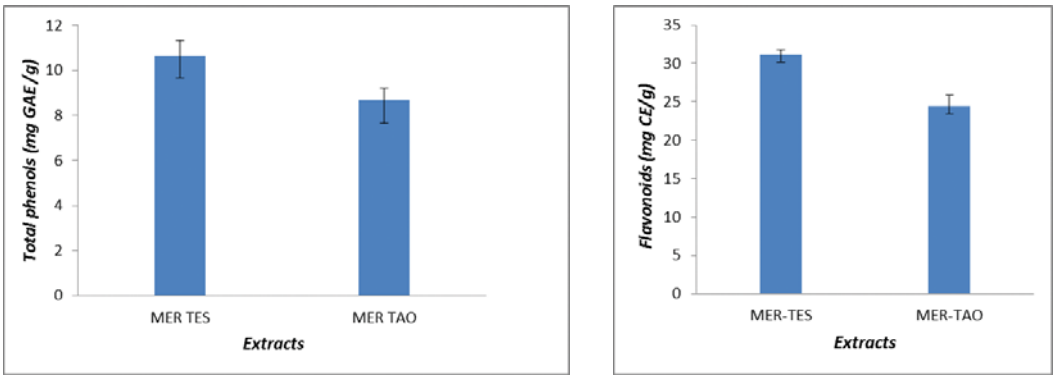

Fig. 2: Total phenols and flavonoids contents of *Ruta chalepensis* leaves *extracts*

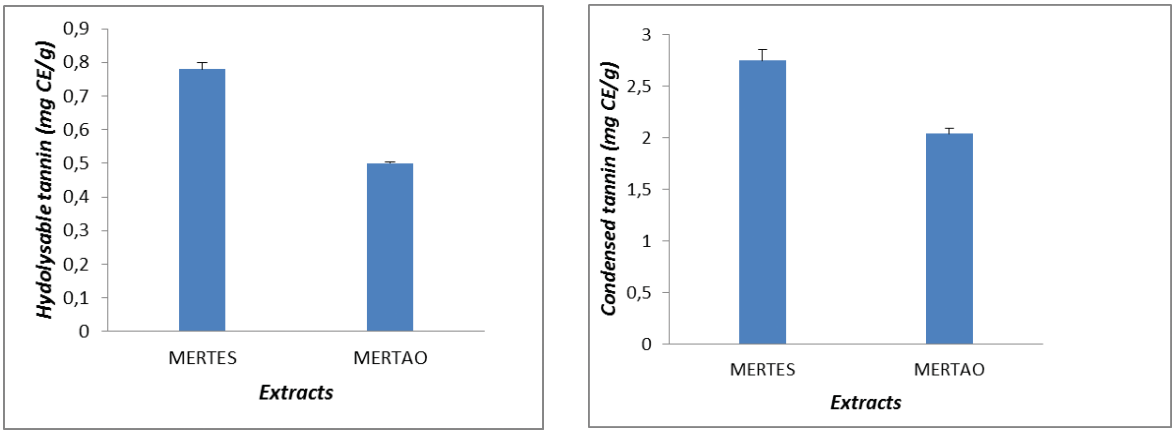

Fig. 3: Hydrolyzable and condensed tannin content of *Ruta chalepensis* leaves *extracts.*

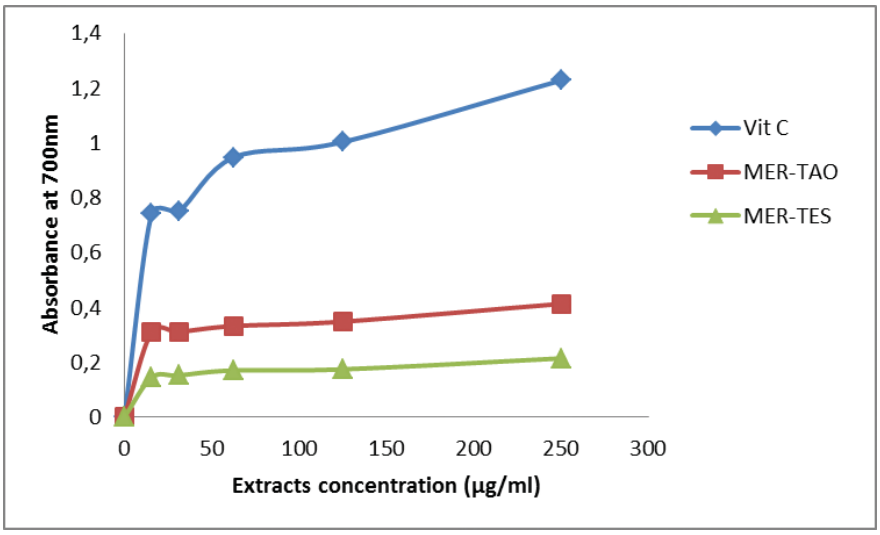

Fig. 4: Antiradical activity of *Ruta chalepensis methanolic extracts*

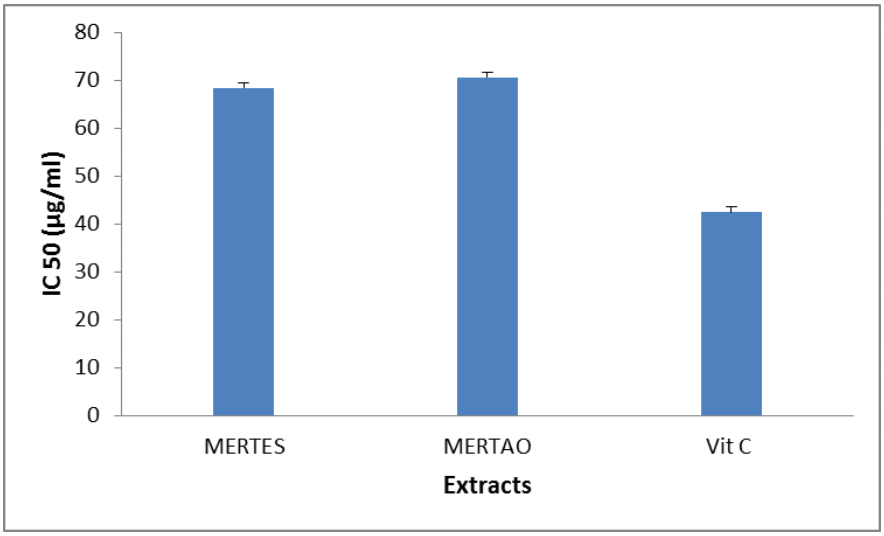

Fig. 5: Median inhibitory concentration of *Ruta chalepensis extracts*

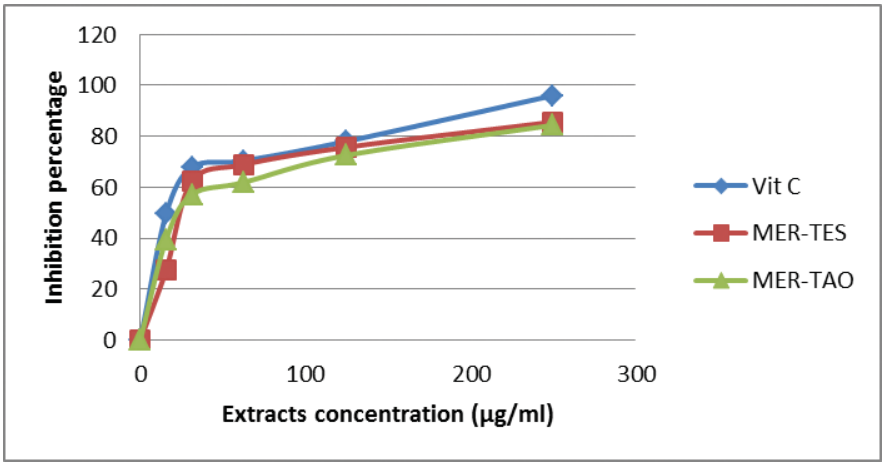

Fig .6: Antiradical activity potency of *Ruta chalepensis extract*

as: the *extract* concentration, the evaluation method, the antioxidant sensitivity to the test temperature, the antioxidant's water -soluble or lipid-soluble nature (Kadri, 2011 and Pukalskas, 2012).

CONCLUSION

From *in vitro* data it was concluded that the *Ruta Chalepensis extract* from the Tessala mountains was slightly rich in certain secondary metabolites and antioxidant elements, than that of the Taougrite mountains. The study has highlighted the influence of area harvest factor of plant on the *extracts* content and quality. In addition, an evaluation of the antioxidant activity of the *Ruta chalepensis* essences against DPPH and FRAP, and the spectral assay of the bioactive substances (polyphenols, flavonoids and tannins), showed that this *Rutaceae* exhibit an interesting antioxidant power suggesting their use in the food, in cosmetics and in the pharmaceutical industry.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest with respect to the publication of this document.

REFERENCES :

Abou-Zeid, H.M. Bidak, L.M. and Goh, Y.M. 2014. Phytochemical screening and antimicrobial activities of some wild medicinal plants of the western Mediterranean coastal region Egypt. *Int. J. Pharm. Sci. Res.*,**5** (10): 4445-4455.

Al-Brashdi, A.S. Al-Ariymi, H., Al Hashmi, M. and Khan, S.A. 2016. Evaluation of antioxidant potential, total phenolic content and phytochemical screening of aerial parts of a folkloric medicine, *Haplophyllum tuberculatum* (Forssk) A Juss. *J. Coast. Life Med*., **4**(4): 315-319.

Al-Farsi, M.A and Lee ,C.Y. 2008. Optimization of phenolics and dietary fibre extraction from date seeds. *Food Chem.,* **108** (3): 977-985.

Al-Said, M.S., Tariq, M.M., Al-Yahya, A., Rafatullah, S., Ginnawi, O.T. and Ageel, A.M .1990. Studies on *Ruta chalepensis*, an ancient medicinal herb still used in traditional medicine. *J Ethnopharmacol*., **28**(3):305-312.

Atmani, D., Chaher, N., Barboucha, M., Ayouni, K., Lounis, H., Boudaoud, H., Debbache, N. and Atmani, D. 2009. Antioxydant capacity and phenol content of selected Algerian medicinal plants. *Food Chem.,* **112** (2):303-309.

Benhammou, N., Atik Bekkara, F. and Kadifkova, P.T. 2009.Antioxidant activity of *methanolic extracts* and some bioactive compounds of *Atriplex halimus*. *Comptes Rendus Chimie*., **12** (12): 1259-1266.

Bentabet, N., Boucherit-Otmani, Z. and Boucherit, K. 2007. Composition chimique et activité antioxydante d'extraits organiques des racines de *Fredolia aretioides* de la région de Béchar en Algérie. *Phytotérapi* .,**12** :364-371.

Bettaieb, A. Moujahed, N. and Ksouri, R. 2012.Secondary compounds characterization in some autochtonous species from a North-Eastern region of Tunisia. Options mèditerranèennes: New approaches for grassland research in a context of climate and socio-economic changes Series A. Séminaires Méditerranéens., n° **102**, Samsun (Turkey).

Bhowmik, D.2019. Aloe vera-Gift to mankind. *International Journal of Minor Fruits, Medicinal and Aromatic Plants,* **5** (1): 01-06.

Bourgaud, F., Gravot, A., Milesi, S. and Gontier, E. 2001. Production of plant secondary metabolites: A historical perspective. *Plant Sci*., **161**(5): 839-851.

Bouzouita, N., Kachouri, F., Ben Halima, M.K. and Chaabouni, M.M. 2008. Composition chimique et activités antioxydante, antimicrobienne et insecticide de l'huile essentielle de *Juniperus phoenicea*. *Journal de la Société Chimique de Tunisie.,* **10** : 119-125.

Chaibeddra, Z., Baaziz, N. and Zellagui, A. 2016. *Ruta tuberculata* Forsk: Phytochemical screening, total phenolic contents and antioxidant activity of crude coumarins and alkaloids extracts. *Der Pharm Lett*., **8** (6):212-216.

Chung, Y.C., Chang, C.T., Chao, W.W., Lin, C.F. and Chou, S.T. 2002. Antioxidative activity and safety of the 50 *ethanolic extract* from red bean fermented by *Bacillus subtilis* IMR-NK1. *J Agric food chem*., **50** (8):2454-2458.

Conti, B., Leonardi, M., Pistelli, L., Profeti, R., Ouerghemmi, I. and Benelli, G . 2013. Larvicidal and repellent activity of essential oils from wild and cultivated *Ruta chalepensis L. (Rutaceae)* against *Aedes albopictus Skuse (Diptera: Culicidae*), an arbovirus vector. *Parasitol Res*., **112**(3) :991-999.

Daoudi, A., Hrouk, H., Belaidi, R., Slimani, I., Ibijbijen, J. and Nassiri, L. 2016. Valorization of *Ruta montana* and *Ruta chalepensis*: Ethnobotanical study, phytochemical screening and Antibacterial activity. *J Mater Environ Sci.*, **7** (3):926-935.

Djeridane, A., Yous, M., Nadjemi, B., Boutassouna, D., Stocker, P. and Vidal, N. 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem*., **97** (4): 654-660.

El Sayed, K., Al-Said, M. S., El-Feraly, F. S. and Ross, S. A. 2000. New Quinoline Alkaloids from *Ruta chalepensis. J. Nat. Prod.*, **63**(7):995-997.

Fakhfakh, N., Zouari, S., Zouari, M.M., Loussayef, C. and Zouari, N. 2012. Chemical composition of volatile compounds and antioxidant activities of essential oil, *aqueous* and *ethanol extracts* of wild Tunisian *Ruta chalepensis L. (Rutaceae). J Med Plant Res*., **6**(4): 593-600.

Ghazghazia, H. Chediab, A., Abderrazakb, M. and Brahima, H. 2013. Comparaison des contenus en polyphénols et de l'activité antioxydante des extraits méthanoliques de quatre plantes collectées du nord de Tunisie. *Microbiol Hyg Alim*., Vol **25**, N° 73 (4ème Journée GEDIV).

Gonzalez-Trujano, M.E. Carrera, D. Ventura-Martinez, R. Cedillo-Portugal, E and Navarrete, A.2006. Neuropharmacological profile of an *ethanol extract* of *Ruta chalepensis* L. in mice. *J Ethnopharmacol*., **106** (1): 129-135.

Gunaydin, K. and Savci, S. 2005. Phytochemical studies on *Ruta Chalepens* (LAM.) LAMARCK. *Nat. Prod. Res.*, **19**(3): 203-210.

Haddouchi, F., Chaouche, T.M., Zaouali, Y., Ksouri, R., Attou, A. and Benmansour, A. 2013. Chemical composition and antimicrobial activity of the essential oils from four *Ruta* species growing in Algeria. *Food Chem.*, **141** (1):253-258.

Hnatyszyn, O., Arenas, P., Moreno, A.R., Rondina, R. and Coussio, J.D. 1974. Plantas reguladoras de la fecundidad segun la medicina folklorica. *Revista de la Sociedad Cientifica*., **14**: 37.

Hayouni, E., Abedrabba, M., Bouix, M. and Hamdi, M. 2007. The effects of solvent and extraction method on the phenolic contents and biological activities in vitro of Tunisian *Quecus coccifera* L. and *Juniperus phoenicea* L. fruit extracts. *Food Chem*., **105** (3): 1126-1134.

Julkunen-Tiitto, R. 1985. Phenolic constituents in the leaves of northern willows: Methods for the analysis of certain phenolics .*J Agric food chem*., **33** (2): 213-217.

Kacem, M., Kacem, I., Simon, G., Ben Mansour, A., Chaabouni, S., Elfeki, A. and Bouaziz, M. 2015. Phytochemicals and biological activities of *Ruta chalepensis* L. growing in Tunisia. *Food Biosci*., **12** (1):73-83.

Kadri, A., Zarai, A., Bekir, A., Gharsallah, N., Damak, M. and Gdoura, R. 2011. Chemical composition and antioxidant activity of *Marrubium vulgare L.* essential oil from Tunisia. *Afr J Biotechnol*., **10**(9): 3908-3914.

Kang, D.G., Yun, C.K. and Lee, H.S. 2003. Screening and comparison of antioxidant activity of extracts of herbal medicines used in Korea. *J. Ethnopharmacol*., **87** (2-3): 231-236.

Kar, A. 2007. Pharmaocgnosy and Pharmacobiotechnology. Revised-Expanded Second Edition. New Age International Limted Publishres, New Delhi. pp. 332-600.

Khlifi, D., Sghaier, R.M., Amouri, S., Laouini, D., Hamdi, M. and Bouajila, J. 2013. Composition and anti-oxidant, anti-cancer and anti-inflammatory activities of *Artemisiaherba-alba, Ruta chalpensis L.* and *Peganum harmala L. Food Chem Toxicol*., **55**: 202-208.

Kim, D.O., Chun, O.K., Kim, Y.J., Moon, H.Y. and Lee, C.Y. 2003. Quantification of polyphenolics and their antioxidant capacity in fresh plums. *J Agric Food Chem*., **51** (22):6509-6515.

Ksouri, R., Megdiche, W., Falleh, H., Trabelsi, N., Boulaaba, M., Smaoui, A. and Abdelly, C. 2008. Influence of biological, environmental and technical factors on phenolic content and

antioxidant activities of Tunisian halophytes. *C R Biol*., **331**(11) :865-873.

Maataoui, B.S.,Hmyene, A. and Hilali, S. 2006.Activités anti-radicalaires d'extraits de jus de fruits du figuier de barbarie (*Opuntia ficus indica*). *Leban Sci J*., **7**(1):3-8.

Majheniè, L. Škerget, M. and Knez, Ž 2007. Antioxidant and antimicrobial activity of guarana seed extracts. *Food Chemistry,* **104**(3);1258-1268.

Mejri , J., Abderrabba, M. and Mejri, M. 2010. Chemical composition of the essential oil of *Ruta chalepensis* L: influence of drying, hydro-distillation duration and plant parts. *Ind. Crops Prod*., **32**(3) : 671-673.

Merghache, S., Hamza, M. and Tabti, B. 2009. Etude phytochimique de l'huile essentielle de *Ruta chalepensis* L. de Tlemcen, Algérie. *Afr Sci*., **5**(1): 67-81.

Mole, S. and Waterman, P.G. 1987. A critical analysis of techniques for measuring tannins in ecological studies: I. Techniques for chemically defining tannins. *Oecologia.,* **72,** (1):137-147.

Molyneux, P. 2004. The use of the stable free radical diphenyl picryl hydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. Sci. Technol.*,**26** (2):211-219.

Ouerghemmi, I., Rebey, I. B., Rahali, F.Z., Bourgou, S., Pistelli, L., Ksouri, R., Marzouk, B. and Tounsi, M.S. 2016. Antioxidant and antimicrobial phenolic compounds from extracts of cultivated and wild-grown Tunisian *Ruta chalepensis*. *J Food Drug Anal*., **25**(2):350-359.

Oyaizu, M. 1986. Studies on products of browning reactions: Antioxidative activities of product of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition and Dietetics*, **44**(6): 307-315.

Pukalskas, A., Venskutonis, P.R., Salido, S., Waard, P. and Van Beek, T.A. 2012. Isolation, identification and activity of natural antioxidants from horehound (*Marrubium vulgare* L) cultivated in Lithuania. *Food Chem.,* **130** (3):695-701.

Qusti, S., Abo-Khatwa, A.N. and Lahwa, M. A. 2010. Screening of antioxidant activity and phenolic content of selected food items cited in the holly Quran. *Eur J Biol Sci*., **2** (1):40-51.

Raaman, N., Natarajan, P., Swaminathan, A., Balan, L. and Laxmanan, H. 2014. *In vitro* propagation, qualitative phytochemical analysis and antioxidant activity of *Ruta chalapensis* L. *Med Plants*, **6** (2):87-93.

Raghav, S.K., Gupta, B., Agrawal, C., Goswami, K. and Das, H.R. 2006.Anti-inflammatory effect of *Ruta graveolens* L. in murine macrophage cells. *J Ethnopharmocol*., **104** (1-2):234-239.

Sabitha Rani, A., Hajera Sana, G., Sulakshana, E., Shravya, P. and Keerti, M. 2019. *Spilanthes acmella*-an important medicinal plant. *International Journal of Minor Fruits, Medicinal and Aromatic Plants*, **5** (2): 15-26.

Shehadeh, M.B., Afifi, F.U. and Abu-Hamdah, S.M. 2007. Platelet aggregation inhibitors from aerial parts of *Ruta chalepensis* grown in Jordan. *Integr Med Insights,* **2** (1): 35-39.

Shuib, N.A., Iqbal, A., Akmal Sulaiman, F., Razak, I. and Susanti, D. 2015. Antioxidant and antibacterial activities of *Ruta angustifolia extract*. *J Teknol (Sciences and Engineering),* **77**(25):101-105.

Turkmen, N., Velioglu, Y. S., Sari, F. and Polat, G. 2007. Effect of extraction conditions were total polyphenol content and antioxidant Measured and antibacterial activities of black tea. *Molecules*, **12** (3):484-496.

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Preharvest fruit bagging time regulates postharvest quality and shelf life of dragon fruit (*Hylocereus* spp.)

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ABSTRACT

Appropriate bagging time is imperative for effective use of fruit bagging technology in safe fruit production. A study was conducted at the Germplasm Centre of Bangladesh Agricultural University (BAU-GPC), Mymensingh during May 2018 to September 2019 with a view to determining the effect of preharvest fruit bagging time on postharvest qualities and shelf life of dragon fruit. The two-factor experiment was comprised of two varieties viz., BAU dragon fruit-1; BAU dragon fruit-2 and three bagging time viz., Fruit bagging at 7, 12 and 17 days after fruit setting (DAFS). The experiment was laid out in randomized complete block design with three replications. Black polythene bag was used as bagging material for this study. It was observed that fruit bagging at 7 DAFS significantly increased fruit length (11.74 cm), flesh weight (188.59 g), moisture content (85.06%), total soluble solids (14.79^{0}Brix) and shortened the maturity time (21.66 days) of BAU dragon fruit-1. Besides, fruit bagging at 7 DAFS significantly increased fruit diameter (7.60 cm), edible rate (74.29%), pH (4.72), dry matter content (20.59%), shelf life (11.65 days) and reduced peel weight (62.57 g), peel thickness (0.22 cm) of BAU dragon fruit-2. From the findings of this study it can be concluded that fruit bagging at 7 days after fruit setting resulted the improve the postharvest quality and shelf life of dragon fruit.

***Keywords*:** Dragon fruit, dry matter content, flesh-peel ratio, peel thickness, pH, TSS

INTRODUCTION

Dragon fruit (*Hylocereus* spp.) is considered as one of the most beautiful fruits of the Cactaceae family with its light red skin stubbed with green scales and white, pink as well as red flesh with tiny black seeds. Dragon fruit is a sprawling or vine, terrestrial or epiphytic cactus which has received worldwide recognition, first as an ornamental plant and then a fruit crop. It is a long day plant with beautiful night blooming flower that is nick named as Nobel women, Queen of the night or the moonflower (Nandi *et al*., 2019). The creamy pulp with edible seeds has a very delicate aroma. The edible black seed of dragon fruit is a good source of omega-3, omega-6 fatty acids, polyunsaturated fats which are healthy fatty acids (Sinha *et al*., 2018). Dragon fruit is one of the most nutritious fruits that increase the digesting power. Besides, it has many medicinal and therapeutic properties as like blood fruit (Kalkame *et al*., 2018) which includes ability to control obesity, cancer, diabetes, high cholesterol as well as high blood pressure as like. Currently, it has been cultivated in many districts of Bangladesh especially, Natore, Pabna, Mymensingh, Gazipur, Munshiganj, Magura and at different Horticulture Centers under the Department of Agricultural Extension (DAE).

Although dragon fruit is heat loving, it can be damaged by long periods of intense sun and heat, resulting in sunscald. Bagging is the best option for protecting dragon fruit from sunburn and fruit cracking. Dragon fruit may also attract ants, beetles and fruit flies. These insects can also hamper the production of this fruit. Dragon fruits are also damaged by birds severely when it's getting mature. Due to various fungi, insects and birds attack, the superior fruit size as well as skin color is not possible get to properly thus infested fruits are not generally sold in the market.

Bagging is a physical protection technique, commonly applied to many fruits, which not only improves their visual quality by promoting peel coloration and reducing the incidence of fruit cracking and rusting, but can also change the micro environment for fruit development, which can have multiple effects on internal fruit quality (Son and Lee, 2008). Bagging has been extensively used in several fruits crops to improve skin color, reduce

the incidence of disease, insect pests, mechanical damage, sunburn of the skin and bird damage and to increase market value.

Due to its many beneficial effects, fruit bagging has become an integral part of different fruits cultivation in many countries of the world. Now-a-days, fruit bagging has been an eco-friendly practice in many kind of fruit such as mango (Hossain *et al.,* 2020), guava (Rahman *et al.,* 2018), banana (Rubel *et al.,* 2019), papaya, citrus, grape etc. However, very limited information has been found on the effect of fruit bagging time of dragon fruit in Bangladesh. Therefore, this experiment was undertaken to find out an optimum bagging time for dragon fruit production in Bangladesh which will ensure postharvest quality and enhance the shelf life of dragon fruit.

MATERIALS AND METHODS

The experiment was conducted at Bangladesh Agricultural University Germplasm Centre (BAU-GPC), Mymensingh, during May 2018 to September 2019. The experimental site was located between 24.43°N latitude, 90.25°E longitude and 18m altitude from the sea level. The soil of the experimental area was sandy loam type and belonging to the old Brahmaputra Flood Plain Alluvial Tract of AEZ 9 having non calcareous dark grey flood plain soil. The selected area was a medium high land. It was fertile and well drained and slightly acidic with p^H varying from 5.4 to 6.7. During the study period, the average maximum and minimum temperature were 34.01^{0}C and 25.30^{0}C, respectively. While the average relative humidity was 85.61%.

The two-factor experiment was conducted following Randomized Complete Block Design (RCBD) with three replications. The experimental treatments were two varieties of Dragon fruit *viz.,* V_1: BAU dragon fruit-1 (White flesh) and V_2: BAU dragon fruit-2 (Red flesh), and three bagging time *viz.,* T_1: Fruit bagging at 7 days after fruit setting (DAFS), T_2: Fruit bagging at 12 DAFS and T_3: Fruit bagging at 17 DAFS.

Regular observation during flowering time was continued to find out fruit setting time after anthesis and fruits were tagged for recording days after fruit setting. Black polythene bag was used for bagging of selected fruits in the month of May, 2019. The fruits were wrapped by black polythene bag at 7, 12 and 17 days after fruit setting. A small portion of two corners of each bag was cut off in order to prevent water deposition inside the bag. The bags were tightly tied with the help of rope so that water and insect-pest could not enter into the bag. Fruits were harvested at full mature stage, a common index of maturity is skin color change to almost full red (Nerd *et al.,* 1999). Five fruits were randomly selected from each replication of each treatment and counted days required to maturity (days). Fresh weight was recorded immediately after harvesting of fruits thereafter fruit length and diameter were measured using digital slide calipers. Fruit peel and flesh were separated carefully and recorded the weight for calculation of flesh-peel ratio and % edible rate. The peel thickness was measured by using digital slide calipers.

Determination of moisture and dry matter content

Fifty grams (50g) of fresh fruit sample of each treatment was taken and cut into small pieces on an aluminum foil and oven dried at 70^{0}C until the constant weight was attained. Percent moisture content was calculated according to the following formula:

$$\% \text{ moisture content} = \frac{\text{Fresh weight of sample(g)} - \text{dry weight of sample(g)}}{\text{Fresh weight of sample(g)}} \times 100$$

% dry matter content was calculated as % dry matter content =100 - %moisture content

Determination of fruit pH

The pH of dragon fruit was recorded by using an electric pH meter. The pH meter was standardized using buffer solutions described by Ranganna (1994). Samples of 10 g fresh pulp was homogenized in 10 ml de-ionized water pH 7.0 and the flesh of homogenate was measured with the pH meter.

Determination of Total Soluble Solids (^{0}Brix)

Total soluble solids (TSS) content of dragon fruit was estimated using digital refractometer (Model N-1 á, Atago Company Ltd., Japan). A drop of juice

was squeezed from the dragon fruit flesh and taken on the prism of refractometer. TSS content was recorded from the direct reading of the instrument. Temperature correction was made using the temperature correction chart.

Shelf life (days)

Five fruits of each variety and treatment were stored in an ambient condition (30±2^{0}C) to observe the storage life. Fruits were monitored regularly and the shelf life of fruits was counted from the date of harvesting to the last edible stage.

Statistical analysis

The collected data on various parameters were statistically analyzed using MSTATC statistical package program. The means for all the treatments were calculated and analysis of variances (ANOVA) for all the parameters was compared by least significant difference (LSD) test at 5% and 1% levels of probability (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Effect of variety on fruit maturity time and morphological traits of dragon fruit

Fruit growth, other developmental traits and time to fruit maturity were impacted differently due to variety during the study period. It was observed that no significant difference found on days to maturity and fruit flesh weight (Table 1) while other studied parameters significantly influenced by fruit variety. In respect of fruit fresh weight, length and peel weight, significant variations were observed between two varieties. The higher fruit fresh weight (246.92 g), length 10.80 cm) and peel weight (86.79 g) were recorded from BAU dragon fruit-1,while BAU dragon fruit-2 produced the lower fruit fresh weight (237.59 g), length (9.01 cm) and peel weight (76.80 g) (Table 1). Similarly, fruit diameter and peel thickness also influenced by the fruit variety. The higher fruit diameter (6.81 cm) and peel thickness (0.36 cm) were recorded from BAU dragon fruit-2, while BAU dragon fruit-1 produced lower fruit diameter (6.53 cm) and peel thickness (0.26cm) (Table 1). The findings of this research are corroborated with the results of Chowdhury *et al.* (2020). They noticed that white fleshed BAU dragon fruit-1 performed superior in terms of fruit length, fresh and peel weight. Similar results were reported by Mallik *et al.* (2018) in white flesh dragon fruit.

Fruit qualitative traits were also influenced by variety. It was found that BAU dragon fruit-2 produced maximum flesh-peel ration (2.15), edible rate (66.84%), dry matter content (17.80%), fruit pH (4.65), TSS (12.79%) and shelf life (9.99 days), while BAU dragon fruit-1 contained highest moisture (85.01%), lowest pH (4.48), TSS (12.41%) and shelf life (9.68 days) (Table 2). Significant variations were noticed on morphological and nutritional traits of two varieties of dragon fruit (Mallik *et al.,* 2018).

Table 1: Effect of variety on days to fruit maturity and morphological traits of dragon fruit

Variety	Days to maturity	Fruit fresh weight (g)	Fruit length (cm)	Fruit diameter (cm)	Flesh weight (g)	Peel weight (g)	Peel thickness (cm)
V_1 (BAU dragon fruit-1)	25.16	246.92	10.80	6.53	159.50	86.79	0.26
V_2 (BAU dragon fruit-2)	24.91	237.59	9.01	6.81	160.15	76.80	0.36
$LSD_{(0.05)}$	0.59	1.27	0.19	0.09	0.94	0.76	0.02
Level of significance	NS	**	**	**	NS	**	**

** indicates significant at 1% level of probability, NS: Non-significant

Effect of bagging time on fruit maturity time and morphological traits of dragon fruit

Fruit bagging time significantly influenced on days required to fruit mature as well as other fruit growth parameters. It was found that fruit bagging at 17 DAFS took the longest time to mature (27.16 days) and the earliest fruit maturity achieved (21.3 days) when fruits were bagged at 7 DAFS (Table 3). The highest fruit fresh weight, longest fruit length, diameter and flesh weight (258.3 g, 11.06 cm, 7.57 cm and 185.63 g, respectively) were obtained from fruits bagging at 7 DAFS and the

Table 2: Effect of variety on flesh-peel ratio, edible rate, moisture content, dry matter content, pH, TSS and shelf life of dragon fruit

Variety	Flesh-peel ratio	Edible rate (%)	Moisture content (%)	Dry matter content (%)	pH	TSS (^{0}Brix)	Shelf life (days)
V_1 (BAU dragon fruit-1)	1.85	64.33	85.01	14.98	4.48	12.41	9.68
V_2 (BAU dragon fruit-2)	2.15	66.84	82.19	17.8	4.65	12.79	9.99
$LSD_{(0.05)}$	0.02	0.23	0.37	0.37	0.05	0.13	0.16
Level of significance	**	**	**	**	**	**	**

** indicates significant at 1% level of probability

lowest fruit fresh weight, fruit length, diameter and flesh weight (240.66 g, 9.55 cm, 6.72cm and 160.52 g, respectively) noticed from fruits bagging at 17 DAFS (Table 3). Costa *et al.* (2017) reported that bagging does not alter the physical and chemical traits of red pitaya. Fruit bagging with different materials did not alter fruit length, diameter, fresh weight and pulp yield of atemoya and sugar apple (Pereira *et al.,* 2009), while Santos *et al.* (2007) achieved higher fresh weight of apple cv. Fiju Suprema from waxed paper bagged fruits.

On the contrarily, early fruit bagging reduced peel weight and thickness resulting the minimum peel weight and thickness (72.04 g and 0.25 cm) obtained from the fruits bagging at 7 DAFS while the maximum peel weight and thickness (85.02 g and 0.31cm) attained from bagging at 12 DAFS (Table 3).

Table 3: Effect of bagging time on days to fruit maturity and morphological traits of dragon fruit

Treatment	Days to maturity	Fruit fresh weight (g)	Fruit length (cm)	Fruit diameter (cm)	Flesh weight (g)	Peel weight (g)	Peel thickness (cm)
T_1 (Bagging at 7 DAFS)	21.83	258.3	11.06	7.57	185.63	72.04	0.25
T_2 (Bagging at 12 DAFS)	24.66	257.29	10.32	7.10	171.64	85.02	0.31
T_3 (Bagging at 17 DAFS)	27.16	240.66	9.55	6.72	160.52	79.50	0.27
$LSD_{(0.05)}$	0.84	1.79	0.27	0.13	1.32	1.08	0.03
Level of significance	**	**	**	**	**	**	**

** indicates significant at 1% level of probability

As fruit flesh weight, peel thickness and peel weight were impacted by fruit bagging time therefore, flesh-peel ratio, percent edible rate and quality of dragon fruit were also affected by the time of bagging. The maximum flesh-peel ratio, edible rate, dry matter content, pH and TSS (2.61, 71.97%, 17.76%, 4.70, 14.34^{0}Brix) obtained from fruits bagging at 7 DAFS and the minimum results recorded from 12 and 17 DAFS (Table 4). Early bagging may increase fruit edible rate as the insect pest infestation remains minimum during that time which was supported by Tran *et al.* (2015). Bagging fruits after 7 days anthesis enhanced % TSS of dragon fruits (Tuan *et al.,* 2017). They stated that about 15% TSS increased due to earlier bagging. In this study we found about 29% improvement of TSS bagging at 7 DAFS. Bentley *et al.* (1992) claimed that sweetness in apple fruit was remarkably enhanced by fruit bagging at the golf-ball size of fruit development.

However, fruit bagging at 17 DAFS contained the highest moisture content (83.36%) but shelf life was the lowest (9.92 days) as compared to other bagging time. The longest shelf life of fruits noticed from fruits bagging at 7DAFS (11.25 days) which had lowest moisture content (82.23%)(Table 4). Fruit bagging effectively protect fruits from any physical injuries, diseases pest attack, bird attack, sunburn and other environmental hazards as a results shelf life of harvested fruits were longer shelf life as compared to non-bag fruits (Huixae, 2010; Hossain *et al.,* 2020). In this study, it was noticed that dragon fruit bagged at 7 DAFS prolonged shelf life as compared to fruit bagging at later stage.

Table 4: Effect of bagging time on flesh-peel ratio, edible rate, moisture content, dry matter content, pH, TSS and shelf life of dragon fruit

Treatment	Flesh-peel ratio	Edible rate (%)	Moisture content (%)	Dry matter content (%)	pH	TSS (Brix)	Shelf life (days)
T_1 (Bagging at 7 DAFS)	2.61	71.97	82.23	17.76	4.70	14.34	11.25
T_2 (Bagging at 12 DAFS)	2.02	66.69	83.05	16.94	4.63	12.47	10.58
T_3 (Bagging at 17 DAFS)	2.03	66.66	83.36	16.64	4.50	11.45	9.92
$LSD_{(0.05)}$	0.03	0.23	0.52	0.52	0.07	0.18	0.23
Level of significance	**	**	**	**	**	**	**

** indicates significant at 1% level of probability

Combined effects of variety and bagging time on days to fruit maturity and morphological traits of dragon fruit

The combined effect of variety and bagging time had significant impact on days to fruit maturity, and other morphological traits and shelf life of dragon fruit. The shortest days to maturity (21.66 days) was found when BAU dragon fruit-1 bagged at 7 DAFS and the longest days to maturity (27.33 days) recorded from BAU dragon fruit-2 bagged at 17 DAFS (Table 5). The highest fruit fresh weight and flesh weight (270.72 g and 188.59 g) were found when BAU dragon fruit-1 bagged at 7 DAFS. The lowest values of those traits (236.57 g and 152.50 cm) obtained from BAU dragon fruit-1 with bagging at 17 DAFS (Fig. 1, Table 5). In carambola, fruit bagging with plastic bag at 10 days after full bloom increased fruit weight (Xu *et al.,* 2008). Marble stage reported as a proper bagging time for mango cv. Alphonso (Haldankar *et al.,* 2015). In other variety of mango (cv. Langra) proper bagging time was reported 40-45 days after fruit setting (Islam *et al.,* 2019) and cv. Amrapali 30-45 days after fruit setting (Hossain *et al.,* 2020).

The highest fruit length(11.74 cm) was obtained when BAU dragon fruit-1 bagged at 7 DAFS and the lowest fruit length (8.40 cm) achieved from the combination of BAU dragon fruit-2 bagged at 17 DAFS but fruit diameter was maximum (7.60 cm) in BAU dragon fruit-2 bagged at 7 DAFS (Table 5).

It was also observed that peel weight, thickness and flesh-peel ratio of dragon fruit significantly influenced due to the combined effect of variety and bagging time. The lowest peel weight (62.57 g) and peel thickness (0.22 cm) obtained from BAU dragon fruit-2 bagged at 7 DAFS and the highest peel weight (88.53 g) was found when BAU dragon fruit-1 bagged at 12 DAFS. Similarly, the maximum peel thickness (0.39 cm) was found when BAU dragon fruit-2 bagged at 12 DAFS (Table 5).

The highest flesh-peel ratio (2.92) was found from BAU dragon fruit-2 bagged at 7 DAFS (V2T1) and the lowest result (1.83) obtained from the combination of BAU dragon fruit-1 and 17 DAFS (V1T3) (Fig. 2). It was also observed that the highest edible rate (74.29%) recorded when BAU dragon fruit-2 bagged at 7 DAFS while the lowest edible rate (64.46%)obtained from BAU dragon fruit-1 under bagged at 17 DAFS (Table 6). Early bagging may increase fruit edible rate as the insect pest infestation remains minimum during that time which was supported by Tran *et al.* (2015).

At the same time the highest fruit moisture content (85.06%) obtained when BAU dragon fruit-1 was bagged at 7 DAFS and the lowest value (79.40%) was achieved from the combination of BAU dragon fruit-2 and 7 DAFS (Table 6).

Fruit pH value and dry matter content also influenced by the combined effects of variety and fruit bagging time. The highest pH (4.72), and dry matter content (20.59%) was found from the combination of BAU dragon fruit-2 and 7 DAFS (V_2T_1) (Table 5, Fig. 3). The lowest pH (4.38) was achieved from the combination of BAU dragon fruit-1 with 17 DAFS (Table 6). While the lowest dry matter content (14.93%) was noticed from the combination of BAU dragon fruit-1 with 7 DAFS (V_1T_1) (Fig.3).

Table 5: Combined effects of variety and bagging time on days to fruit maturity and morphological traits of dragon fruit

Treatment	Combinations	Days to maturity	Fruit length (cm)	Fruit diameter (cm)	Flesh weight (g)	Peel weight (g)	Peel thickness (cm)
V_1(BAU dragon fruit-1)	Bagging at 7 DAFS	21.66	11.74	7.54	188.59	81.50	0.28
	Bagging at 12 DAFS	24.66	11.40	6.79	165.40	88.53	0.24
	Bagging at 17 DAFS	27.00	10.69	6.41	152.50	83.43	0.22
V_2 (BAU dragon fruit-2)	Bagging at 7 DAFS	22.00	10.37	7.60	182.66	62.57	0.22
	Bagging at 12 DAFS	24.66	9.24	7.41	177.87	81.50	0.39
	Bagging at 17 DAFS	27.33	8.40	7.03	168.54	75.57	0.32
$LSD_{(0.05)}$		1.19	0.39	0.19	1.87	1.52	0.04
Level of significance		**	**	**	**	**	**

** indicates significant at 1% level of probability

Table 6: Combined effect of variety and bagging time on edible rate, moisture content, pH, TSS and shelf lifeof dragon fruit

Treatment	Combinations	Edible rate (%)	Moisture content (%)	pH	TSS (^{0}Brix)	Shelf life (days)
V_1(BAU dragon fruit-1)	Bagging at 7 DAFS	69.66	85.06	4.68	14.79	10.84
	Bagging at 12 DAFS	64.97	83.99	4.58	12.19	9.96
	Bagging at 17 DAFS	64.46	84.58	4.38	10.41	9.85
V_2(BAU dragon fruit-2)	Bagging at 7 DAFS	74.29	79.40	4.72	13.90	11.65
	Bagging at 12 DAFS	68.41	82.10	4.68	12.74	11.21
	Bagging at 17 DAFS	68.86	82.14	4.62	12.49	10.00
$LSD_{(0.05)}$		0.46	0.74	0.09	0.26	0.33
Level of significance		**	**	**	**	**

** indicates significant at 1% level of probability

However, the highest TSS (14.79 Brix) was found when BAU dragon fruit-1 bagged at 7 DAFS and the lowest TSS (10.41 Brix) achieved from the combination of BAU dragon fruit-1 and 17 DAFS (Table 6). The longest shelf life (11.65 days) was obtained from BAU dragon fruit-2 with bagging at 7 DAFS and the shortest shelf life (9.85days) recorded when BAU dragon fruit-1 bagged at 17 DAFS (Table 6).

However, the highest TSS (14.79%) was found when BAU dragon fruit-1 bagged at 7 DAFS and the lowest TSS (10.41%) achieved from the combination of BAU dragon fruit-1 and 17 DAFS (Table 6). Bentley *et al*. (1992) noticed that sweetness in apple fruit was significantly improved due to bagging at the golf-ball size of fruit development. The longest shelf life (11.65 days) was obtained from BAU dragon fruit-2 with bagging at 7 DAFS and the shortest shelf life (9.85days) recorded when BAU dragon fruit-1 bagged at 17 DAFS (Table 6). Chowdhury *et al* (2020) reported that fruit bagging with black polythene bag extended shelf life of dragon fruit as it protects fruits from all insect pests infestation and maintained microenvironment of the fruit. Fruit bagging at 7 DAFS greatly enhanced fruit maturity,

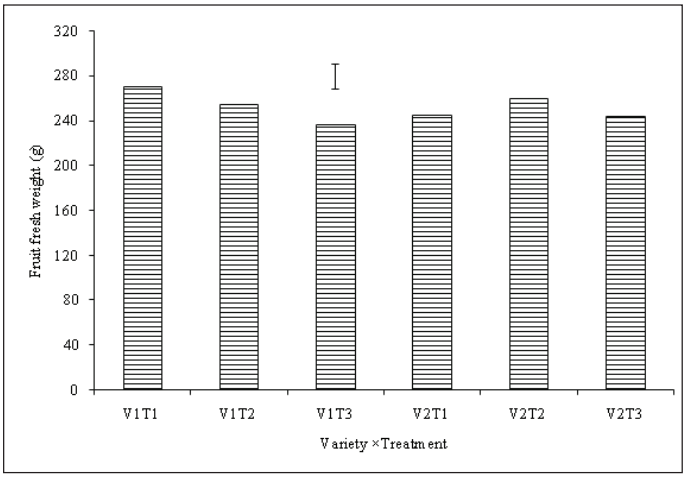

Fig. 1: Combined effect of variety and treatment on fruit fresh weight of dragon fruit. Bar indicates LSD at 1% level of probability. V_1: BAU dragon fruit-1, V_2: BAU dragon fruit-2, T_1: Bagging at 7 DAFS, T_2: Bagging at 12 DAFS, T_3: Bagging at 17 DAFS.

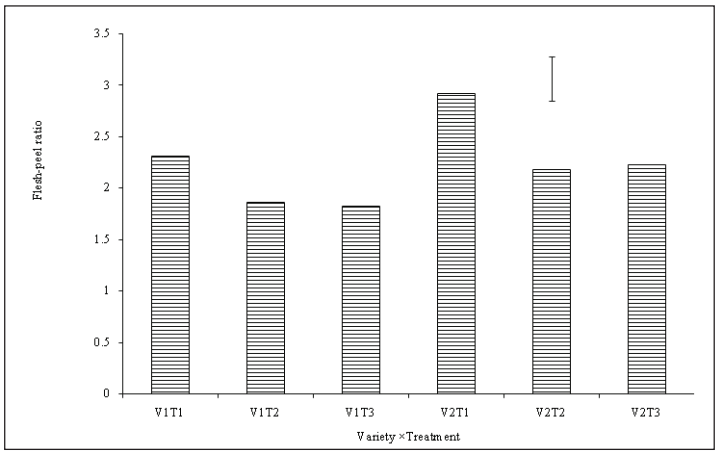

Fig. 2: Combined effect of variety and treatment on flesh-peel ratio of dragon fruit. Bar indicates LSD at 1% level of probability. V_1: BAU dragon fruit-1, V_2: BAU dragon fruit-2, T_1: Bagging at 7 DAFS, T_2: Bagging at 12 DAFS, T_3: Bagging at 17 DAFS.

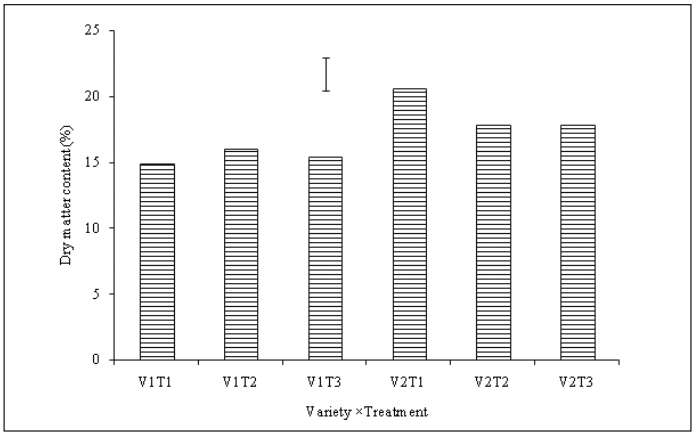

Fig. 3: Combined effect of variety and treatment on dry mater content of dragon fruit. Bar indicates LSD at 1% level of probability. V_1: BAU dragon fruit-1, V_2: BAU dragon fruit-2, T_1: Bagging at 7 DAFS, T_2: Bagging at 12 DAFS, T_3: Bagging at 17 DAFS.

fruit weight, flesh weight, edible rate and remarkably reduced peel thickness and increased flesh-peel ration. These findings are in agreement with the results of Tuan *et al.* (2017). They stated that bagging fruits after 7 days anthesis produced highest fresh weight, edible rate as compared to bagging after 15 days anthesis as well as non-bagged fruits.

CONCLUSION

Considering the findings of this experiment, it can be concluded that both the varieties showed better results in terms of fruit quality and shelf life while fruit bagging at 7 days after fruit setting. Fruit bagging with black polythene bag promoted almost all the morphological traits of fruit as well as days required to maturity, total dry weight, dry matter content, TSS, pH and shelf life of dragon fruit. It can be summarized that fruit bagging at 7 days after fruit setting with black polythene bag could be an effective eco-friendly technology to produce quality dragon fruit with longer postharvest storage life.

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REFERENCES :

Bentley, W.J. and Viveros, M. 1992. Brown-bagging 'Granny Smith' apples on trees stops codling moth damage. *California Agric*., **46**:30–32.

Chowdhury, S., Hossain, M.M. and Rahim, M.A. 2020. Postharvest quality and shelf life of dragon fruit (*Hylocereus* spp.) as influenced by preharvest fruit bagging materials. *J. Agric. Food Environ.,* **1(4):** 33-40.

Costa, A. C., Ramos, J. D., De Menezes, T. P., Laredo, R. and Duarte, M. H. 2017. Quality of Pitaia fruits submitted to field bagging. *Revista Brasileira de Fruticultura*, **39**, (e-277) DOI: 10.1590/0100-29452017 377

Gomez, K.A. and Gomez, A.A. 1984. Statistical Procedures for Agricultural Research. *Wiley and Sons Interscience*, New York. pp. 67-215.

Haldankar, P.M., Parulekar, Y.R., Kireeti, A., Kad, M.S., Shinde, S.M. and Lawande, K.E. 2015. Studies on influence of bagging of fruits at marble stage on quality of mango cv. Alphonso. *J. Plant Studies,***4(2)**: 12-20.

Hossain, M.J., Hossain, M.M., Rabbani, M.G., Hafiz, M.M.H. and Islam, M.Z. 2020. Effects of preharvest fruit bagging on postharvest quality and shelf life of mango cv. Amrapali. *J. Bangladesh Agril. Uni.,***18(1**): 61–67.

Huixae, H. 2010. High-quality and high efficiency cultivation techniques of subtropical fruit trees. Text book of International Training Workshop 6th edition, Baise, China.

Islam, M. T., Rahman, M. S., Akter, M. M., Hasan, M. N. and Uddin, M. S. 2019. Influence of pre-harvest bagging on fruit quality of mango (*Mangifera indica* L.) cv. Langra. *Asian J. Agril.Hort. Res.,* **4(4)**:1-10.

Kalkame Ch. Momin, Achume, N., Sangma, C. P., Suresh, Singh,Y. S. and Rao, S. R. 2018. Blood fruit [*Haematocarpus validus* (Miers) Bakh. f. ex Forman] – A potential nutraceutical and therapeutic fruit plant. *International Journal of Minor Fruits, Medicinal and Aromatic plants,* **4**(1): 44-49.

Mallik, B., Hossain, M. and Rahim, A. 2018. Influence of variety and flowering time on some physio-morphological and chemical traits of dragon fruit (*Hylocereus* spp.). *J. Hort. Postharvest Res.,* **1(2)**: 115-130.

Nandi, Priyanka, Tarai, R.K. and Ghosh, S.N. 2019. Study on rooting behaviour of different types of cutting of Dragon fruit at different period of year. *International Journal of Minor Fruits, Medicinal and Aromatic plants,* **5**(2):45-49.

Nerd, A., Gutman, F. and Mizrahi, Y. 1999. Ripening and postharvest behavior of fruits of two Hylocereus species (Cactaceae). *Postharv. Biol. Technol.,***17**:39-45.

Pereira, M. C.T., Bandeira, N., Antunes Junior, R.C., Nietsche, S., de Oliveira Junior, M. X., Alvarenga, C.D., Santos, T. M. dos., Oliveira, J. R. 2009. Bagging effect upon either quality of the fruits and incidence of the fruit borer in atemoya and sweetsop. *Bragantia*, **68(2):**389-396.

Rahman, M.M., Hossain, M.M., Rahim, M.A., Rubel, M.H.K. and Islam, M.Z. 2018. Effect

of pre-harvest fruit bagging on post-harvest quality of guava cv. Swarupkathi. *Funda. Appl. Agric.,***3(1):**363-371.

Ranganna, S. 1994. Handbook of analysis and quality control for Fruit and Vegetable Products. Tata McGraw-Hill Publishing Company Ltd., New Delhi, India.

Rubel, M.H.K., Hossain, M.M., Hafiz, M.M.H., Rahman, M.M. and Khatun, R. 2019. Effect of banana bunch covering technology for quality banana production in Bangladesh. *Prog. Agric.,* **30(3)**: 238-252.

Santos, dos J.P., Wamser, A.F. and Denardi, F. 2007. The quality of bagged fruit of different apple genotypes. *Ciencia Rural, Santa Maria*, **37(6):** 1614-1620.

Sinha, R., Jha, M.K. and Karuna, K. 2018. Dragon fruit: A fruit for health benefits and nutritional security. Int. J. Agric. Sci., **8(2):** 97-100. Doi:1024247//ijasrapr2018014.

Son, I.C. and Lee, C.H. 2008. The effects of bags with different light transmittance on the berry cracking of grape 'Kyoho'. *Hort. Environ. Biotechnol.,***49(2):**98-103.

Tran, D.H., Yen, C.R. and Chen, Y.K. 2015. Effects of bagging on fruit characteristics and physical fruit protection in red pitaya (*Hylocereus* spp.). *Biol. Agric.Hort.,***31(3):**158-166.

Tuan, N.M., Hung, N.T., Anh, B.L. and Hung, N.V. 2017. Effect of bagging time on fruit yield and quality of red pitaya (*Hylocereus* spp.) fruit in Vietnum. *Intl. J. Plant Soil Sci.,***19(6):**1-7.

Xu, C.X., Chen, H.B., Huang, R.Y. and He, Y.J. 2008. Effects of bagging on fruit growth and quality of carambola. *Acta Hort.,***773**: 195–200.

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Development of novel fairness oil formulated from selected medicinal plants

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ABSTRACT

There is a drug group in Ayurveda medicine which is indicated for discoloration of the skin. It is correlated with removing of pigmentations, skin dryness and improving the skin tone cosmetically. A study was aimed to prepare novel oil by using the method of oil preparation from selected drugs of the said group. Further, this study was focused to establish basic standardization procedures on new formulated oil to add scientific validity and analyzed the pharmacological actions of selected drugs. The oil was prepared in three methods in accordance with the authentic text to obtain the high yield and quality. Organoleptic parameters, phytochemical parameters and physico-chemical parameters were applied to establish the standardization parameters.

The most predominant Ayurveda pharmacological actions of the drugs were Kapha-pitta Shâmaka action and increase of complexion of skin. Physico-chemical parameters of oils were as follows: pH value of A, B, C samples were: 5.46, 5.88, 6.55 respectively. Acid value of A, B, C samples were: 5.049mg of KOH, 5.610mg of KOH, 6.732mg of KOH respectively. Presence of alkaloids, tannins, saponins, flavonoids and terpenoids could be seen in all samples which were useful in the quality control and standardization of novel oil. Fairness effect could be proven conceptually via Ayurveda pharmacological actions of oil and scientifically via its wide range of phytochemical components as well as modern pharmacological findings such as tyrosinase inhibitors and Alpha and beta hydroxyl acids and derivatives. In conclusion, this novel oil would provide safe and effective action on skin complexion. However, to validate its therapeutic utility to scientific community, preclinical and clinical studies are essential.

***Keywords*:** herbal drugs, complexion, standardization

INTRODUCTION

The fair skin concept has been made psychological and social impact on women which is considered to be affected on their personality. Recently men also concerned about fairness and handsomeness with the market promotions. As the demand on these cosmetics have elevated, it withholds the market at higher stand. Besides the busy lifestyle schedules, consumption of instant food, exposure to the polluted/unhealthy environment, and the bright sun, the discoloration of skin and the features of aging could be seen commonly among the people. Hence the high expenditure on skin care among the population has led to many more skin problem of pigmentations, loss of complexion and skin dryness (Kamakshi, 2012).

Prevailing whitening products in today market are containing of heavy metals, bleaching chemical agents, which are harmful. Hence recently, world trend turns to use medicinal plant products in health care system. Global need of herbal medicine has resulted in growth of natural product markets and improvement in traditional systems of medicine.

According to Ayurveda philosophy skin complexion is maintained by the *Pitta Dosha*. When *Pitta Dosha* is decreased and vitiated, the skin complexion is also diminished. *Bhrâjaka Pitta* is the accountable subtype of the *Pitta Dosha* for the maintenance of body complexion and it removes the pigmentation and discoloration of the skin (Prajnâsârabhidhâna, 1990). The moistness of the skin is wielded by the *Susneha* (oily) and *Drava* (liquidity) qualities of *Pitta*.

Novel "phytoactive" ingredients are revealing the development in cosmeceuticals and these ingredients include many beneficial properties in skin caring. Hence this study is based on the overcoming of current problems regarding skin complexion by inventing novel oil which was prepared with the use of selected medicinal plants. The selected medicinal plants were *Raktha Chandana* (*Pterocarpus santalinus* L.f.), *Thunga*

(*Calophyllum inophyllum*), *Padma* (*Nelumbo nucifera* Gaertn.), *Ushira* (*Vetiveria zizaniodes* (L.) Roberty), *Madhuka (Glycyrrhiza glabra*L.), *Manjishtâ* (*Rubia cordifolia* L.), *Sârivâ* (*Hemidesmus indicus* (L.) R. Br.), *Payasya* (*Pueraria tuberosa* (Willd.) DC.). Further, establishing of the standardization parameters for the novel oil and then analyze its pharmacological action for the selected medicinal plants were the other aims of this study. Hence, the applicability of new formula is discussed by considering pharmacological properties of them in this study. Further, this study possesses basic standardization procedures of new formulated oil.

MATERIALS AND METHODS

Selection, processing and quality evaluation of the raw materials

All the ingredients (Table 1) were collected cleaned and dried them well. It was then identified macroscopically and studied for important botanical characteristics. All ingredients were authenticated by Department of *Dravyaguna Vignana*, IIM.

Table 1:Collection of raw materials

	Used part	Sanskrit name	Botanical name
For *Kalka* (paste) and	Pith	*Raktha Chandana*	*Pterocarpus satalinus*
Drava Dravya	Bark	*Thunga*	*Calophyllum inophyllum*
(liquid portion)	Stalk	*Padma*	*Nelumbo nucifera*
	Root	*Ushira*	*Vetiveria zizaniodes*
	Root	*Madhuka*	*Glycyrrhiza glabra L.*
	Root	*Manjishtâ*	*Rubia cordifolia*
	root	*Sârivâ*	*Hemidesmus indicus*
	Tubers	*Payasya*	*Pueraria tuberosa*
SnehaDravya(oil portion)	Oil	*Nârikhela*	*Cocos nucifera*
Kalka (paste) for *Thaila*	Outer cover of fruit	*Nimbu*	*Citrus medica*
Mûrchana(special	Leaf stem	*Thâmbula*	*Piper betle*
processing)	Seed	*Krushnajîraka*	*Nigella sativa*

Due to the unavailability of *Padma kâshta*, stalk of lotus (*Nelumbo nucifera*) was used as a substitute (Department of Ayurveda, 1994).

Preparation of *Kalka* (paste): *Kalka Paribhâshâ* of the Ayurveda drug preparation was followed to prepare the *Kalka* (Nagodavithana P, 2001).The coarse powder of dry ingredients was used to prepare the *Kalka* (Wijerathna, 2017).

Preparation of *Kashaya* (Decoction): *Kashâya* was prepared by adding 120gof ingredients and 1920ml of water and simmered to 480ml (Nagodavithana, 2001).

Preparation of *Thaila* (oil): Paste: oil: liquid portion (Decoction) were mixed in the standard proportions to prepare the oil samples (Department of Ayurveda, 1994).

Sample A – The oil is heated up to *MadyaPâka* (Nagodavithana, 2001).

Sample B – The oil is heated up to *KharaPâka* (Nagodavithana, 2001).

Sample C – *NârikhelaThailaMurchana* (Nagodavithana, 2001) was done to the base oil. The oil is prepared by heating up to *Khara Pâka*.

Three repetitions were done to each 3 samples.

Phytochemical analysis of the *Kashâya* (Decoction)

Test for Alkaloids: Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's reagent was added to the mixture. Appearance of cream colour precipitate indicates the presence of Alkaloid (Varughese and Tripathi, 2013).

Test for Tannins: Crude extract was mixed with 2ml of neutral $FeCl_3$. A dark green colour indicates the presence of Tannins Alkaloid (Varughese and Tripathi, 2013).

Test for Saponins: Crude extract was mix with 5ml of distilled water in a test tube and shaken vigorously. The formation of stable foam taken as an indication for the presence of saponins Alkaloid (Varughese and Tripathi, 2013).

Test for Terpeneoids: Crude extract was mixed with 2ml of chloroform and concentrated H_2SO_4 was added sideways. A reddish brown colour at the interface indicates the presence of terpenoids Alkaloid(Varughese and Tripathi, 2013).

All the tests were done 3 times for each sample.

Analytical study of the *Thaila* (oil)

Organoleptic characters: The oil samples were inspected for the differences of colour, odour, appearance, texture, and touch in the four samples.

p^H Value: p^H meter was used to determine the P^H of four samples with threerepetitions for each.

Moisture content: Hot plate method was used to evaluate moisture content. Weighed quantity of oil was taken in a glass beaker, heated on the electric hot plate until the cessation of the rising bubbles of steam as well as the absence of foam. After cooling, it was reweighed. The difference in the weight, before and after heating was used to calculate the amount of moisture presents (loss on drying) (BIS, 2007). This was done 3 times for each sample and the values were declared as percentages.

Acid value: Weighed quantity of oil was taken and added 50ml of neutralized ethyl alcohol and 1ml of phenolphthalein indicator solution into it. The mixture was boiled for 5 minutes and titrated while as hot as possible with standard potassium hydroxide (0.1N)solution, shaking vigorously while titration (BIS, 2007). Three Repetitions were done for each sample.

$$\text{Acid value} = \frac{56.1\ VN}{W}$$

Where,

V = Volume in ml of standard KOH solution used,

N = Normality of standard KOH solution,

W = Weight in g of the oil taken.

Peroxide value: Weighed quantity of oil was dissolved in 30ml of glacial acetic acid: chloroform (3:2) solution. 0.5ml of saturated potassium iodide solution was added to it. After shaking it one minute, 30ml of distilled water was added. Then it was titrated with 0.1N sodium thiosulphate solution with constant and vigorous shaking, using starch solution as the indicator, near the end point (BIS, 2007).The procedure was repeated for the 3 samples.

$$\text{Peroxide value} = \frac{(S-B) * N * 1000}{g}$$

Where,

S = Volume in ml of sodium thiosulphate solution used up by the sample,

B = Volume in ml of the sodium thiosulphate solution used up in the blank determination,

N = Normality of the sodium thiosulphate solution,

g = Weight in g of the sample

Analysis of the pharmacological actions of the ingredients: Analysis of pharmacological actions of the ingredients was done (Department of Ayurveda, 1994).

RESULTS

Phytochemical analysis of *Kashâya* (Decoction)

Table 2:Phytochemical analysis of *Kashâya*(Decoction)

Phytochemical	Sample A	Sample B	Sample C
Alkaloids	+	+	+
Tannins	+	+	+
Saponins	+	+	+
Terpenoids	+	+	+

Organoleptic properties of oil

Table 3: Organoleptic properties

Organoleptic properties	Sample A	Sample B	Sample C
Colour	Reddish orange	Red	Red
Odour	Odour of coconut oil	Odour of coconut oil	Odour of coconut oil
Appearance	Transparent	Transparent	Transparent
Texture	Liquid consistency	Liquidconsistency	Liquidconsistency
Touch	Oily	Oily	Oily

Physico – chemical parameters

pH Value

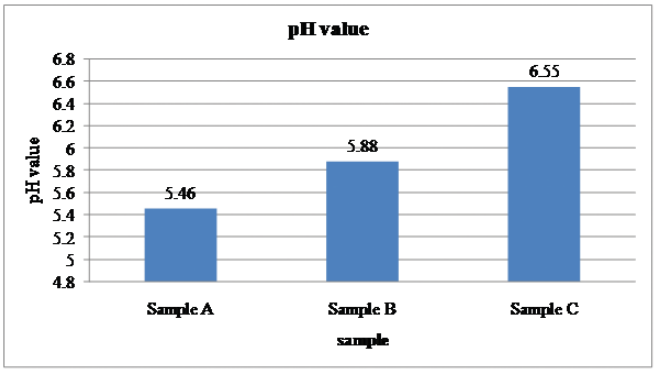

Figure 1: Varying of the pH value of samples.

Sample A- oil heated up to *MadyaPâka*,

Sample B- oil heated up to *KharaPâka*,

Sample C- oil prepared with *Mûrchitha* coconut oil and heated up to *KharaPâka*

The highest pH value is observed in sample C. The average pH value is 5.9.

Moisture content

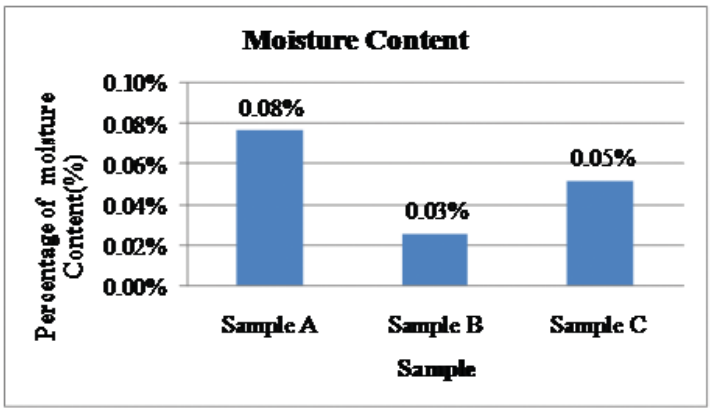

Fig. 2: Differentiation of moisture content of oil samples.

Sample A- oil heated up to *MadyaPâka*,

Sample B- oil heated up to *KharaPâka*,

Sample C- oil prepared with *Mûrchitha* coconut oil and heated up to *KharaPâka*

Highest percentage of moisture was presented in sample A and the lowest was in sample B. The average moisture content is 0.045%.

Acid value

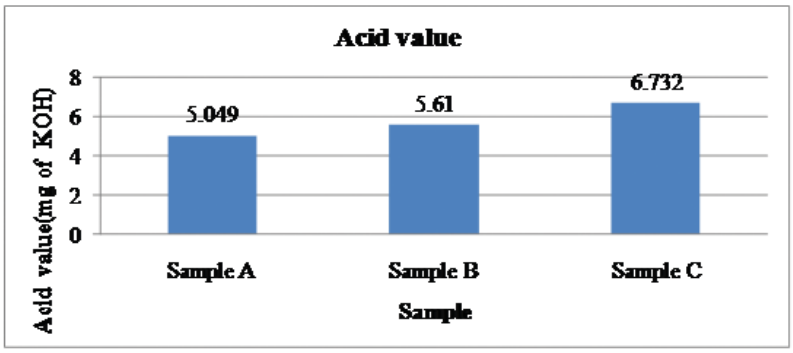

Fig. 3: Differentiation of Acid values of oil samples.
Sample A- oil heated up to *MadyaPâka*,
Sample B- oil heated up to *KharaPâka*, Sample
C- oil prepared with *Mûrchitha* coconut oil and heated up to *KharaPâka*
The highest acid value was presented in sample C and lowest was in sample A. The average acid value is 5.797

Peroxide value

Peroxide value couldn't be determined because of the first colour change is difficult to observe due to the red colour of the oil.

Final volume of oil samples

Table 4:Final volumes

Sample	Initial volume	Final volume	Percentage
Sample A	120ml	100ml	83.3%
Sample B	120ml	75ml	62.5%
Sample C	120ml	70ml	58.3%

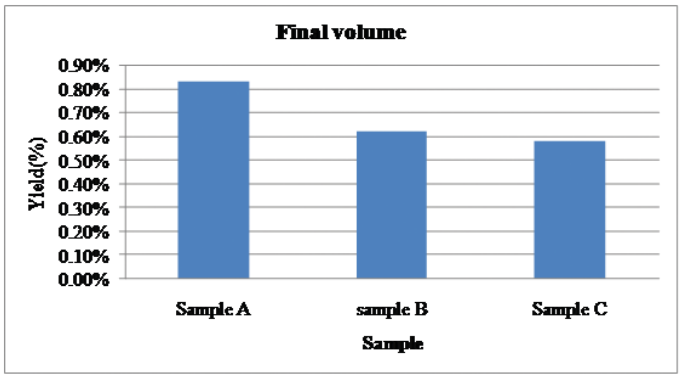

Fig. 4: Varying of the final volumes of oil samples
Sample A- oil heated up to *MadyaPâka*,
Sample B- oil heated up to *KharaPâka*,
Sample C- oil prepared with *Mûrchitha* coconut oil and heated up to *KharaPâka*.
Highest yield was obtained from sample A and lowest was obtained from sample C.
Pharmacological actions according to Effecton Dosha and External therapeutic action

Table 5: Pharmacological properties according to effects on *Dosha* and external therapeutic action

Ingredients	Effect on *Dosha*	External therapeutic action
Raktha Chandana (Pterocarpus satalinus)	Pacify *Kapha, Pitta*	Pacify burning sensation, relieve disorders of skin, relieve swellings
Thunga(Calophyllum inophyllum)	Pacify *Kapha, Pitta*	Scraping action, analgesic action
Padma(Nelumbo nucifera)	Pacify *Kapha, Pitta*	Increase complexion, pacify burning sensation
Ushira(Vetiveria zizaniodes)	Pacify *Kapha, Pitta*	Pacify burning sensation, relieve disorders of skin
Madhuka(*Glycyrrhiza glabra L.)*	Pacify *Vâta, Pitta*	Pacify burning sensation, relieve swellings, beneficial on hair
Manjishtâ(*Rubia cordifolia)*	Pacify *Thridosha*	Pacify burning sensation
Sârivâ(Hemidesmus indicus)	Pacify *Thridosha*	Pacify burning sensation, relieve swellings
Payasya(*Pueraria tuberosa*)	Pacify *Vâta, Pitta*	Increase complexion
Sithâ (controversial)	Pacify *Vâta, Pitta*	-
Durvâ(Cynodon dactylon)	Pacify *Thridosha*	Pacify burning sensation

Pitta – Kapha Shâmaka effect, increasing complexion and Pacify burning sensation actions prominent in *Varnya Dashaka*.

DISCUSSION

In this research to prepare the novel oil, the drug group which is called *Varnya Dashakaya* is used as the base formula which is mentioned in Ayurveda authentic text, *Charaka Samhithâ*. Based on the availability and non-controversial, herbs were selected for the novel oil and techniques were followed for the manufacturing and quality evaluation of oil. Hence 08 drugs of the oilwere used for the preparation except *Sithâ* and *Durvâ* due to its controversiality. Some authentic text have mentioned *Sithâ* as *Shvetha Durvâ* (Kumârasinghe, 1991) and some as sugar cane*(Sharkarâ)* (Department of Ayurveda,1994). *Durvâ* has mentioned as *Krushna Durvâ/ Nil Îthana* (Kumârasinghe,1991) and it was difficult to find. Also Lotus stalks (*Nelumbo nucifera*) were used as a substitute for *Padmakâshta* (Department of Ayurveda,1994). The commonly used part of each drug was used for the preparation.

Decoction of the 08 drugs was used as *Drava Dravya (Liquid portion)* for the preparation of oil. The modified proportion of methodology of preparation of decoction was used which is mentioned in an Ayurveda text book, *Shârangdhara Samhithâ* in order to get the high yield of oil.

Coconut oil was used as the base oil as it is rich in nutrients such as vitamin E, as well as with good essential oils. Conversely, based on its nutritional and pharmacological effects coconut oil has been identified as best for all the types of skin and its common in availability. It has anti-fungal, anti-bacterial and anti-viral properties that make it beneficial for the immune support (Patil, 2018). Thus coconut oil itself possesses advantageous in skin complexion and anti-aging effects and adding of the drugs of *Varnya Dashakaya* would enhance its effectiveness. At the same time *Varnya Dashakaya* provided an attractive colour and fragrant to the oil.

Mûrchana is a special process that is commonly used in Ayurveda oil preparation to enrich its efficacy, quality and shelf life. Therefore to assess the validity of *Mûrchitha Thaila* in this kind of preparation Sample C was prepared by using *Mûrchitha* Coconut oil (Department of Ayurveda,1994).

Phytochemical studies were done only for the *Kashâya* (Decoction) because the same drugs were used to *Kalka* (Paste) and *Kashâya* (Decoction).The oil was consisted with phytochemicals such as Alkaloids, Tannin, Terpenoids, Saponin. Hence, the oil has anti-bacterial, anti-fungal and anti-oxidant properties which will be beneficial in skin complexion as well as in providing protection from pathogenic bacteria and fungi.

The reddish colour of oil is due to the *Manjishtâ (Rubia cordifolia)* and *Raktha Chandana (Pterocarpus satalinus)*which are ingredients of *Kalka*(Paste) and *Kashâya* (Decoction). Characteristic odour is due to the coconut oil which used as base oil and pleasant aroma was presented due to herbs.

The average pH is 5.9 and this average pH is beneficial and safe for skin (Lambers, 2006) as the normal skin pH is about 4.5 – 5.5.

The average moisture content of oil is 0.045%. Highest percentage observed in sample A (0.077%) as it was prepared up to *Madya Pâka*. Hence this average percentage is beneficial for the persistence of shelf life of the oil.

Standard acid value of coconut oil is about 6.00g/mg of KOH (Auriga Research, 2013). Although the sample C is bit higher than reference range still all the samples are in the required range. An acid value is associated with the shelf life of the oils and it is the measure of free fatty acids of oil. It is an indicator of inadequate processing and storage conditions (i.e., high temperature and relative humidity).

Good average of yield (68%) can be seen in final volume in all samples due to use of the coarse powder for *Kalka Dravya* (paste).

In Ayurveda, process of formation of skin in foetus is attributed to *Pâka* of *Raktha Dhâthu*. *Agni Mahâ Bhûta* (heat) is said to be at the root of formation of complexion (Trikamji, 2005). There are 7 layers of skin according to the Ayurveda and the first layer which is called as *Avabhâsinî* is the responsible layer for the complexion of the body. *Brâjaka Agni/ BrâjakaPitta* have located in the first layer (Prajnâsârabhidhâna, 1990). This helps to make the lustre and complexion of body.

When *Pitta Dosha* gets vitiated, the action of *Brâjaka Pitta* is diminished. Then luster and complexion is decreased and discolorations are occurred. The *Âma* of skin is increased and *Kapha Dosha*gets vitiated. Hence unctuousness of skin and skin tone get diminished.

According to the modern science skin-lightening ingredients can also be classified by their source, such as classes to which they belong. The important classes are;

i. Chemical tyrosinase inhibitors (hydroquinone and similar type of compounds)
ii. Botanicals (essentially from plants and algae)
iii. Anti-oxidants
iv. Vitamins – A, B, C, E
v. Peptides
vi. Alpha and beta hydroxyl acids and derivatives

Among them botanical extracts mostly contains a combination of two or more classes of compounds that works synergistically to achieve skin lightening. Botanicals connote nature and are hence more acceptable to people (Kmakshi, 2012).

According to Ayurveda, the herbs which alleviate *Pitta*, *Raktha* and also *Kapha* in general either acting through their *Rasa, Vipâka* or *Prabhâva* considered as *Varnya*. This oil has *Madhura, Tiktha, Kashâya Rasa, Guru, Snigdha Guna, Shîtha Vîrya* and *Madhura Vipâka* which is pacify the *Pitta Dosha* and has *Tiktha* and *Kashâya Rasa* which are pacify *Kapha Dosha* and retain the action of *Varnya.*

Hence this oil is capable in improving skin complexion, luster, moistness and skin tone.

This Oil is developed for the first time as a novel product for the given drug list and would be beneficial in skin complexion which will be popular among the beauty conscious people. The pharmacodynamics properties proved its capability as a cosmetic product. However further studies are essential to develop this as a marketed product.

REFERENCES :

Auriga Research Pvt.ltd. 2013.Oils [http://foodsafteyhelpline.com/2013/03/oils/] Accessed 21 July 2018.

Ayurveda Aushadha Samgraha. 1994. Sinhala Edition: Vol 1;Part 2;Department of Ayurveda, Colombo, Sri Lanka, p110.

Ayurveda Aushadha Samgraha. 1994. Sinhala Edition: Vol 1;Part 1;Department of Ayurveda, Colombo, Sri Lanka, p35.

Ayurveda Aushadha Samgraha. 1994. Sinhala Edition: Vol 1;Part 2;Department of Ayurveda, Colombo, Sri Lanka, p36.

Ayurveda Aushadha Samgraha. 1994. Sinhala Edition: Vol 1;Part 3;Department of Ayurveda, Colombo, Sri Lanka, pp60- 321.

Ayurveda Aushadha Samgraha. 1994. Sinhala Edition: Vol 1;Part 1;Department of Ayurveda, Colombo, Sri Lanka, p14.

BIS, 2007. Sampling, Physical and Chemical tests, Indian standard methods of sampling and test for oils and fats: Part 1, Bureau of Indian Standards, New Delhi, India, p20

BIS, 2007. Sampling Physical and Chemical tests; Indian standard methods of sampling and test for oils and fats: Part 1, Bureau of Indian Standards, New Delhi, India, p29

BIS, 2007. Sampling, Physical and Chemical tests, Indian standard methods of sampling and test

for oils and fats: Part 1, Bureau of Indian Standards, New Delhi, India, p.64

Kmakshi, R. 2012. Fairness via Formulation: A Review of Cosmetic Skin- lightening Ingredients: *Journal of Cosmetic Science*, **63**(1): 46.

Kumârasinghe A.1991. *Caraka Samhitâ, Sûtra Sthâna*: Sinhala Edition: Vol 1; Department of Ayurveda, p29.

Lambers, H., Piessens, S., Bloem, A., Pronk, H. and P. Finkel. 2006. Natural Skin Surface $_{p}$H is on avarage below 5, which is beneficial for its resident flora, Pub Med, *International Journal of Cosmetic Science*, **28**(5): 359-370

Nâgodavithâna, P. 2001. Sharângdhara Samhitâ, Madhyama Khanda: Sinhala Edition: Samayawardhana Publications, Colombo, Sri Lanka, p171.

Nâgodavithâna, P. 2001, Sharângdhara Samhitâ, Madhyama Khanda: Sinhala Edition: Samayawardhana Publications, Colombo, Sri Lanka, p172

Nâgodavithâna, P. 2001. Sharângdhara Samhitâ, Madhyama Khanda: Sinhala Edition: Samayawardhana Publications, Colombo, Sri Lanka, p123

Patil K. 2018. Properties of Coconut oil: Organic Information services, India.

Prajnâsârabhidhâna K.S. 1990. Susruta Samhitâ, Sharîrasthâna*:* Sinhala Edition: Vol 3; Department of Ayurveda, Colombo, Sri Lanka, p149

Prajnâsârabhidhâna K.S. 1990. Susruta Samhitâ, Sharîrasthâna**:** Sinhala Edition*:* Vol 3; Department of Ayurveda, Colombo, Sri Lanka, p151

Trikamji AY, 2005. Susruta Samhitâ: Varanasi Chaukhamba Oriantalia, Varanasi, India, p348

Varughese, B and Tripathi J. 2013. Phytochemical Evaluation of different solvent extracts of *Aelgle marmelos* fruit at different stages of its ripening. *Advances in Life Science and Technology*, **8**: 8-12.

Wijerathna K.M.C.H. 2017. Establish the standards of Roma Nashaka Karaviradi Tailaya (Not published). Chapter 2. Institute of Indeginouse Medicine, University of Colombo, Sri Lanka. p21

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Fruit physico-chemical studies of some local wood apple (*Limonia acidissima* L.) genotypes

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ABSTRACT

Considering the importance of wood apple fruit, physico-chemical properties of twelve selected local genotypes have been studied. Within various fruit physical characters such as its shape, size, fruit weight, pulp weight, rind weight and rind thickness, the Genotype 11 and Genotype 4 had found to have maximum values. Fruits were found of the shape of flattened round, round, and oblong. Fruit length and diameter ranged from 7.9cm to 10.7cm and 7.1cm to 9.8cm, respectively. Fruit weight has been ranged from 97.4g to 225.7g, while pulp weight ranged from 50.7g to 117.5g. Average seed weight varied from 6.5g to 37.9g counting 56 to 531.6 numbers of seeds. Though variation in rind thickness was lesser, however, rind weight exhibited from 53.45g to 78.40g. With respect to quality of the fruits, maximum TSS was recorded in Genotype 4 (22.08°Brix) and minimum in Genotype 10 (11.16°Brix). Highest total sugar was noticed in Genotype 9 (2.83%) and reducing sugar was maximum in Genotype 6 (1.73%). Fruits of different genotypes exhibited acidity ranging from 0.70 to 2.33% while ascorbic acid 7.45 to 24.98 mg/ 100g. From the above results it can be concluded that Genotype 4, 6, 11 were very promising in terms of better size, where as Genotype 1 and 6 were very encouraging for fruit quality characters.

Key words: Bio-chemical characters, genotypes, wood apple

INTRODUCTION

Wood apple (*Limonia acidissima* L.) is one of the lesser known fruit like Roktogota (*Haematocarpus validus)* in Bangladesh (Rahim *et al*., 2015) and considered as one of the most important plant of 'Ayurveda'. It is one of the important minor fruit in South East Asia with immense nutritional and therapeutic potential. Various important phyto-constituents like alkaloids, phenolic compounds, triterpenoids, coumarins, tannins, steroids etc. have been isolated from the fruit. Being the rich source of vitamins and minerals it is used as a stomachic, diuretic, cardiotonic and tonic and very recently reported its use in gastrointestinal disorders. This fruit belongs to family Rutaceae having chromosome number 2n = 18 and is commonly known as curd apple, monkey fruit, elephant apple, kavat, curd fruit and kath bel in India (Mazumder *et al*., 2006).

There are no such improved types or recognized varieties of wood apple in India. The semi-arid lateritic belt of West Bengal has the rich diversity of wood apple genotypes. But no report of the studies on fruit physic-chemical properties of such diversified genotypes available in this zone. Although the popularity and the demand of this underutilized fruit crop is very high, the production of this fruit is very meager due to non availability of recognized superior types. In the absence of suitable cultivars, expected growth in production of this crop has not been accomplished till date. Identification of suitable genotypes, therefore, becomes imperative for promoting its production, productivity and quality. Thus the present study has been conducted to assess the diversity of some wood apple genotypes under semi-arid lateritic belt of West Bengal.

MATERIALS AND METHODS

The study was carried out at the laboratory of Department of Horticulture and Postharvest Technology, Institute of Agriculture, Visva-Bharati, Sriniketan, West Bengal. Birbhum. The diversity rich district of wood apple was explored and fruits of 12 different genotypes (denoted as WA) were collected from different villages of various blocks such as Surul, Ruppur, Bahadurpur, Ballavpur, Sahebdanga, Makarampur, Raipur etc. 30 fruits of

wood apple were randomly selected from all the direction of marked genotypes and the bulk of sample of all the selected trees from each site collected into bags and tagged by the number and subjected to physico-chemical analysis in the departmental laboratory. The observation on three replicates of samples, each consisting of 10 fruits, the physical characters in terms of fruit shape, size, shell, seed, pulp etc. and qualitative characters in terms of total soluble solids (TSS), total sugar, reducing sugar, titratable acidity and ascorbic acid content analyzed and recorded. The fruit weight was taken on electric weighing balance. The shells were broken and the seeds and pulp were extracted. The total soluble solid content of fruit was determined with the help of hand refractometer (ERMA) of 0 – 32 percent, calibrated at 20 º C. The readings were corrected as per international temperature correction table and the result was expressed in º brix. Total acidity was determined by titrating the diluted fruit juice against 0.1 N NaOH solution using phenolphthalin as an indicator and the results were expressed as percentage fresh weight of fruit. The total sugar content was determined by titrating the diluted fruit juice after hydrolysis with hydrochloric acid against Fehling 'A' and Fehling 'B' solutions in presence of methylene blue as an indicator. The reducing sugar content was determined by titrating the diluted juice against Fehling 'A' and Fehling 'B' solutions by using methylene blue as an indicator. Ascorbic acid content of the fruit was estimated by using 2, 6-dichlorophenol indophenol dye titration method as described in AOAC (1995). The data were statistically analyzed as per method outlined by Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

Fruit physical characters

The perusal of the data presented in Table 1 shows the wide variation of fruit physical characters of wood apple genotypes studied under the experiment. The fruit weight varied from 97.46g in WA-7 to 225.73g in WA-11. Higher fruit weight is a desirable character in wood apple. The fruit length was found maximum in WA-4 (10.77 cm) while minimum was found in WA-7 (7.97 cm). The fruit diameter was recorded maximum in WA-11 (9.80 cm), while minimum diameter was recorded

Table 1: Fruit physical characters of local wood apple genotypes

Genotypes	Fruit shape	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	Pulp weight (g)	Rind weight (g)	Rind thickness (mm)	Weight of seeds/ fruit (g)	Number of seeds/ fruit	Pulp: Seed ratio
WA - 1	FR	8.97	8.63	146.13	76.50	54.00	3.53	15.63	220.2	4.89
WA - 2	R	9.20	9.17	154.00	76.06	63.81	2.96	14.12	213.4	5.37
WA - 3	R	8.43	8.07	180.66	110.00	57.10	2.64	13.56	221.6	8.12
WA - 4	O	10.77	9.77	206.33	112.26	78.40	2.72	15.66	252.7	7.16
WA - 5	O	9.77	8.60	195.13	109.26	66.23	2.66	19.63	321.0	5.57
WA - 6	R	8.33	8.13	177.66	106.46	53.45	2.74	17.75	302.1	6.00
WA - 7	R	7.97	7.90	97.46	50.73	39.49	2.47	7.24	56.3	7.15
WA - 8	O	8.13	7.17	102.60	51.60	44.45	2.40	6.54	73.7	7.89
WA - 9	R	9.47	9.43	173.66	108.40	48.21	2.47	17.05	181.4	6.42
WA - 10	R	8.77	9.17	161.80	81.50	71.22	3.37	9.07	141.5	8.99
WA – 11	R	10.17	9.80	225.73	117.54	70.24	2.59	37.94	531.7	3.09
WA - 12	O	8.87	8.53	184.26	110.30	57.49	3.36	16.47	221.3	6.73
S.E.(m) ±		0.12	0.12	4.03	3.65	1.76	0.06	0.79	9.93	0.28
C.D. at 5%		0.35	0.37	11.92	10.78	5.20	0.18	2.35	29.32	0.79

WA= Wood apple genotypes, FR= Flattened round, R=Round and O= Oblong

Table 2: Fruit bio-chemical characters of local wood apple genotypes

Genotypes	TSS (°Brix)	Acidity (%)	Ascorbic acid (mg/100g)	Total Sugar (%)	Reducing Sugar (%)	TSS : Acidity
WA - 1	22.00	1.13	24.98	2.10	1.54	19.46
WA – 2	21.90	1.00	21.40	1.52	0.82	21.9
WA – 3	18.50	2.06	11.72	1.28	0.89	8.98
WA – 4	22.08	1.51	7.45	1.54	0.85	14.62
WA – 5	18.50	1.31	10.53	1.81	1.38	14.12
WA – 6	17.83	0.70	15.31	2.28	1.73	25.47
WA – 7	19.83	1.86	12.71	2.04	1.43	10.66
WA – 8	17.16	1.25	12.36	1.55	0.97	13.72
WA – 9	18.33	2.34	15.01	2.83	0.90	7.83
WA – 10	11.16	1.60	16.02	2.40	0.83	6.97
WA – 11	18.50	1.80	13.17	1.83	1.02	10.27
WA - 12	18.50	1.79	14.24	1.77	0.93	10.33
S.E.(m)±	0.70	0.10	1.06	0.08	0.05	0.20
C.D. at 5%	2.08	0.31	3.13	0.23	0.14	0.57

WA= Wood apple genotypes.

in WA-8 (7.17 cm). Pulp weight ranged from 50.73 g (WA-7) to 117.54 g (WA-11). Rind weight was found maximum in WA-4 (78.40 g), while minimum was found in WA-7 (39.49 g). Rind thickness ranged from 2.40 mm (WA-8) to 3.53 mm (WA-1) which was measured with help of vernier caliper. Number of fruits varied from 213.5 (WA-3) to 360(WA-12). The different shapes of the fruits like flattened round, round, and oblong was observed. Regarding the total weight of seeds per fruit the maximum was found in WA-11(37.94g) while the minimum was found in WA-8 (6.54g). Total number of seeds per fruit ranged from 56.3 (WA-7) to 531.7 (WA-11). Regarding ratio of pulp weight to seed weight maximum was found in WA-10 (8.99) and minimum was found in WA-11 (3.09). Different fruit shape and size of wood apple were also found by Ghosh *et al.* (2011) and Singh *et al.* (2016).

Bio-chemical characters

Table 2 shows significant variations among the wood apple genotypes with respect to different biochemical parameters. The TSS content varied from 11.16°Brix (WA-10) to 22.08°Brix (WA-4). The titratable acidity was found minimum in WA-6 (0.70 %) and maximum in WA-9 (2.34%). The ascorbic acid content was found highest in WA-1 (24.98 mg/100g) and lowest in WA-4 (7.45mg/100g). The total sugar and reducing sugar was found maximum in WA-9 (2.83%) and WA-6 (1.73%), respectively. The minimum total sugar and reducing sugar was found in WA-3(1.28%) and WA-2(0.82%), respectively. TSS: acidity ratio was found maximum in WA-6(25.47) and minimum in WA-10(6.97). Total soluble solids content of the fruit pulp found in the present experiment has the conformity of findings of Singh *et al.* (2016) where they reported TSS range of 11.07-19.36°Brix. Titratable acidity was found in similar with that found by Sharma *et al.* (2014) where they found ripe fruits were less acidic (1.74%) than both un-ripe (2.92%) and half-ripe (2.40%) respectively. Ascorbic acid content of the fruits was also found similar with the findings of Singh *et al.* (2016) where they observed it in the range from 7.08 - 19.60mg/100 g. Sharma *et al.* (2014) reported the average total sugar content of wood apple fruit pulp around 2.12% and reducing sugar was around 1.23%. Thus the total sugar and reducing sugar content of different wood apple genotypes in the present experiment was in line with the findings of Sharma *et al.* (2014).

CONCLUSION

From the findings of the experiment it can be concluded that there was a wide variability in morphological and physico-chemical characters of

12 wood apple genotypes which were explored. Findings with respect to better fruit size and edible matter show that WA-4, WA-6, and WA-11 were very promising. Related to fruit quality such as TSS, acidity, ascorbic content, total sugar, and reducing sugar the genotypes WA-1 and WA-6 were very encouraging. The information about the nature and magnitude of genetic variability as well as associations among key traits would be helpful in formulating an effective breeding programme for its genetic improvement or genetic upgradation of this valuable crop.

REFERENCES :

AOAC. 1995. *Official Methods of Analysis*. Association of Official Analytical Chemists 16th Ed., Washington, DC, USA.

Ghosh, S.N., Banik, A.K., Banik, B.C., Bera, B., Roy, S., and Kundu, A. 2011. Conservation, multiplication and utilization of wood apple (Feronia limonia)-a semi-wild fruit crop in West Bengal (India). *Proceedings of the 1st International Symposium on Wild Relatives of Subtropical and Temperate Fruit and Nut Crop*, Davis, CA., USA, March 19-23, 2011.

Mazumder, R., Bhattacharya, S., Mazumder, A., Pattnaik, A. K., Tiwary, P. M. and Chaudhary, S. 2006. Antidiarrahoeal evaluation of *Aegle marmelos* (Correa) Linn. root extract. *Phytother Res.*, **20**: 82-84.

Panse, V.G., and Sukhatme, P.V. 1967. Statistical method for agricultural workers. New Delhi: ICAR Pub, pp 153-175.

Rahim, M.A., Khatun, M. J. M., Mahfuzur Rahman, M., Anwar, M.M. and Mirdah, M.H. 2015. Study on the morphology and nutritional status of Roktogota (*Haematocarpus validus)* - an important medicinal fruit plant of hilly areas of Bangladesh. *International Journal of Minor Fruits, Medicinal and Aromatic Plants*, **1**(1): 11-19

Sharma, H.P., Patel, H., Sharma, S., and Vaishali. 2014. Study of Physico-Chemical Changes During Wood Apple (*Feronia limonia*) Maturation. *J. Food Res. Techno*., **2** (4): 148-152.

Singh, A.K., Singh, S., Yadav, V., and Sharma, B.D. 2016. Genetic variability in wood apple (*Feronia limonia*) from Gujarat. *Indian J. Agri Sci.*, **86** (11): 1504–8.

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Study on antimicrobial property of *Agaricus bisporus* (Button Mushroom)

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ABSTRACT

Agaricus bisporus (FCM#03) sp. provides bioactive compounds that claim to posses antibacterial activity. The aim of this research paper is to know antimicrobial activity of various extract of Agaricus bisporus. Fruiting bodies of Agaricus bisporus (FCM#03) were extracted by maceration method using acetone, ethanol, methanol and water. The antimicrobial activity of various solvent extracts (50µg/ml) of Agaricus bisporus were tested against six species of bacteria. The antibacterial activity of methanolic and acetone extract were found to be more Inhibitory effect of these two extract against all bacteria. Fungi toxicity of Agaricus bisporus (FCM#03) was tested by adopting poisoned food technique. The concentrations of extracts, used in the present study were 100, 250, 500 and 1000 mg/ml. The acetone extract was the most effective against. F. oxysporum, C. lunata and M. gypsum which were completely inhibited at 500 mg/ml of concentration.

***Keywords*:** *Agaricus bisporus* (FCM#03), antimicrobial, bioactive compound**.**

INTRODUCTION

A mushroom is defined as "a macro fungus with a distinctive fruiting body which can be either epigeous or hypogenous. The macro fungi have fruiting bodies large enough to be seen with the naked eye and to be picked up by hand" (Chang and Miles, 2004). Edible mushrooms once called the "food of the gods" and still treated as a garnish or delicacy can be taken regularly as part of the human diet or be treated as healthy food or as functional food. The extractable products from medicinal mushrooms, designed to supplement the human diet not as regular food, but as the enhancement of health and fitness, can be classified into the category of dietary supplements/mushroom nutriceuticals (Chang and Buswell, 1996). A nutraceuticals can be defined as a substance that may be considered a food or part of a food that provides medical or health benefits like the prevention and treatment of disease (Cristiane *et al*., 2016). Mushrooms have become attractive as a functional food and as a source for the development of drugs and nutraceuticals (Lakhanpal and Rana, 2005), responsible with their antioxidant, antitumor (Jones and Janardhanan, 2000) and antimicrobial properties. Besides their pharmacological features, mushrooms are becoming more important in our diet due to their nutritional value, related to high protein and low fat / high energy contents (Agahar-Murugkar and Subbulakshmi, 2005). *Agaricus bisporus*, known as table mushroom, cultivated mushroom or button mushroom, is an edible basidiomycete fungus which naturally occurs in grasslands, fields and meadows across Europe and North America. It has spread much more widely and is one of the most widely cultivated mushrooms in the world. Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, and atherosclerosis as well as in degenerative processes associated with aging (Halliwell and Gutteridge, 1984). The aim of present study was to investigate the antimicrobial activity of *Agaricus bisporus*.

MATERIALS AND METHODS

Collection of mushroom sample

Agaricus bisporus (FCM#03) fruiting bodies were collected from Biodiversity Conservation and Rural Biotechnology Centre, Jabalpur (Madhya Pradesh, India). The fungus sample was maintained in PDA (Potato Dextrose Agar) slant. The strains (FCM#03) were used in the present study.

Preparation of the culture media

Nutrient agar media (NAM) (Agrawal and Hasuja, 1986) and Potato dextrose agar (PDA) media were used in the study for antimicrobial analysis.

Organisms used for evaluation of antimicrobial activity

In-vitro antimicrobial susceptibility test were performed using a set of microbes such as Gram negative, Gram positive bacteria and fungi. All bacterial and fungal strains were obtained from Collection Center, Biodiversity, Conservation and Rural Biotechnology Centre, (BCRBC), Jabalpur.

The strains used for antibacterial activity were:

- *Bacillus subtilis* (BCRBC# 1682)
- *Enterococcus faecium**
- *Salmonella paratyphi**
- *Salamonella typhi* (BCRBC# 250)
- *Staphylococcus aureus (*BCRBC#*478)*
- *Klebsiella pneumoniae* (BCRBC# 210)

The strains used for antifungal activity

- *Aspergillus niger* - FCN#34
- *Aspergillus flavus* - FCN #120 (A-4)
- *Fusarium oxysporum* - FCN #80
- *Curvularia lunata* - FCN #62
- *Alternaria alternata* - FCN #120
- *Microsporu M. gypsum* - FCN #I8(MG)
- *Sclerotium rolfsii* - FCN #340

Extraction of bioactive compounds from fruiting bodies of *Agaricus bisporus* (FCM#03):

In the present study all the parts of fruiting bodies of *Agaricus bisporus* (FCM#03) were rinsed separately with distilled water. After that surface sterilized with 70% ethanol and shade dried in the laboratory. Dry mushroom sample were grinded with the help of grinder machine and were stored in sterile, air tight bottles and were used as extraction. 10 gm of *Agaricus bisporus* (FCM#03) powder was subjected to Soxhlet extraction using micro Clevenger type of apparatus for 10 hrs using 100 ml of following solvents viz., ethanol, methanol, acetone and distilled water. Cycles were done 6-10 times and extract was recovered by filtration and extracts were concentrating into 30% by rotavapour for further analysis (Avnish *et al*., 2020)

Filter paper disc diffusion method (FDDM)

Antimicrobial activity of mushroom extract was carried out by following the filter paper disc diffusion technique (Vincent and Vincent, 1944 and Khedoudja *et al.,* 2020 and Paul Njenga *et al*., 2017). This method is also known as Kirby-Bauer method being recommended by the NCCLS (National Committee for Clinical Laboratory Standards). The antimicrobial properties of different extracts were evaluated in different dilutions and the dilution of mushroom extracts were made by dissolving these extracts into its respective solvents.

A small amount (1 ml) of 18 hrs old suspension of each bacterium was then separately added to Erlenmeyer flasks containing 100 ml sterilized and cooled (40°C) nutrient agar medium (NAM). Flasks were gently shaken to mix bacterial cells in the medium. Aliquots of 20ml seeded medium were poured in each sterile petriplates. Sterile filter paper discs (5mm diameter) each impregnated with different dilution of each essentials oil (Methanolic, Ethanolic and Acetone) were placed at equidistance on upper surface of seeded agar medium. The plates were left for 30min at room temperature. Antibiotic disc Gentamycin sulfates (40mg/ml) were used as a positive control, while discs soaked in respective solvent were used as a blank control. The zone of inhibition formed by each extract in different dilution and controls was recorded after 24 hour of inhibition at 35 ± 2°C for bacteria.

Poisoned food technique (PFD)

Grover and Moore (1962) adopted to evaluate the effect of herbal extract on the growth of microorganisms. 20 ml of sterilized and cooled (40°C) growth media (PDA) with desired concentration of antibiotic were poured into pre-sterilized petriplate. Requisite amount of different concentrations of extracts were added into the plates. The assay plates rotated clockwise and anticlockwise to ensure an even distribution of the extract into the medium. In control plates the medium was subjected with respective solvents. After the solidification of agar medium, a disc (5 mm diameter) of test organism from 7 days old culture was placed aseptically in the centre of each plate. The assay plates were incubated at 28+2°C for 7 days. The experiment was run in triplicate.

RESULTS AND DISCUSSION

Antimicrobial activity of different extracts of *Agaricus bisporus* (FCM#03)

Results obtained from disc diffusion method revealed that methanolic extracts of *Agaricus bisporus* (FCM#03) (50 mg/ml) showed maximum activity against *B. subtilis* followed by *S. typhi, S. aureus, E. faecium* and *K. pneumonia*, while acetone extracts showed maximum activity against *S. typhi* and minimum against *S. paratyphi*. Water extract showed weak activity against different bacterial strains. Maximum variations were observed in the activity of acetone extract at desire period of temperature and incubation. The results obtained through filter paper disc diffusion method (FDDM) were more or less similar against same set of microbial strains. Determination of antimicrobial activity of *Agaricus bisporus* (FCM#03) was performed by adopting disc diffusion, well diffusion and poisoned food plate technique, against set of Gram positive and Gram negative bacterial strains. Similar results have also been obtained by Paul Njenga *et al*. (2017) and Andrew *et al*. (2019) regarding antimicrobial activity of mushroom.

Fungi toxic effect by poisoned food technique

Fungi toxicity of *Agaricus bisporus* (FCM#03) was tested by adopting poisoned food technique. The concentrations of extracts, used in the present study were 100, 250, 500 and 1000 mg/ml.

The acetone extract was the most effective against. *F. oxysporum, C. lunata* and *M. gypsum* which were completely inhibited at 500 mg/ml of concentration. While growth inhibition of *A. niger, A. flavus* and *Sclerotium rolfsii* was observed 80.0, 78.5 and 77.7% respectively at 1000 mg/ml of concentration.

Ethanolic extract completely inhibited the growth of *A. flavus* and *S. rolfesii* at 1000 mg/ml, while at the same concentration 91.1, 92.6 and 82% inhibition on growth of *A. niger*, *F. oxysporum* and *M. gypsum* was inhibited respectively but the same concentration did not sufficiently inhibit the growth of *C. lunata and A. alternata*.

Activity of methanolic extract was found to be concentration dependents *M. gypsum* was most susceptible to the methonolic extract 50% reduction in growth was observed at 100 mg/ml while it was 100% reduction at 500 mg/ml 100% reduction in *C. lunata* and *S. rolfssi* was seen against 1000 mg/ml of concentration. Rest of the fungal mycelia of A. *niger, A. flavus, F. oxysporum* and *A. alternata* were not 100% inhibited even at 1000 mg/ml of concentration.

The water extract of the *Agaricus bisporus* was failed to inhibit the 100% growth of any of the fungi which has been taken into present study. Maximally 68.8% of inhibition was observed against *F. oxysporum* at 1000 mg/ml of concentration. The extract was least effective against *C. lunata, A. alternata, S. rolfsi, A. flavus* and *A. niger*. Mycelial growth of *M. gypsum* was reduced by 60.2% at 100 mg/ ml. Daniela *et al*. (2013) reported antifungal peptide designated mushroom which exhibited antifungal activity against pathogenic fungi.

CONCLUSION

The present study described the status of *Agaricus bisporus* (FCM#03) and provided antimicrobial properties and the justification for continuing search for novel drugs. The utilization of *Agaricus bisporus* compound has excellent

Table 1:Evaluation of antimicrobial activity of different extracts of *Agaricus bisporus* (FCM#03).

S. No.	Test organisms	Zone of inhibition (mm)				
		Methanol (50µgml^{-1})	Ethanol (50µgml^{-1})	Acetone (50µgml^{-1})	Distilled water (50µgml^{-1})	Gentamycin sulphate (40µgml^{-1})
1	*Bacillus subtilis*	18.00 ± 0.21	10.60 ± 0.37	21.00 ± 0.00	8.00 ± 0.33	30.20 ± 0.04
2	*Enterococcus faecium*	21.00 ± 0.05	15.20 ± 0.04	20.00 ± 0.18	10.60 ± 0.30	34.50 ± 0.02
3	*Salmonella paratyphi*	14.20 ± 0.08	15.00 ± 0.01	15.00 ± 0.33	7.90 ± 0.08	30.10 ± 0.02
4	*Salamonella typhi*	17.00 ± 0.12	10.00 ± 0.01	20.60 ± 0.19	7.00 ± 0.06	34.00 ± 0.01
5	*Staphylococcus aureus*	18.30 ± 0.20	9.00 ± 0.15	18.00 ± 0.11	8.00 ± 0.04	31.00 ± 0.03
6	*Klebsiella pneumoniae*	21.30 ± 0.08	11.30 ± 0.10	23.60 ± 0.12	8.40 ± 0.02	29.50 ± 0.07

Table 2:Fungitoxic spectrum of *Agaricus bisporus* (FCM#03) extracts measured by poisoned food technique

S. No.	Mushroom extract	Concentration (μgml^{-1})	Percentage of growth inhibition					
			A. Niger	*A.flavus*	*F. oxysporum*	*C. lunata*	*A. alternata*	*M. gypsum*	*S. rolfsii*
1.	Methanolic Extract	100	20.00 ± 0.05	49.00 ± 0.02	28.50 ± 0.07	28.00 ± 0.00	15.90 ± 0.21	50.00 ± 0.10	30.20 ± 0.04
		250	30.90 ± 0.20	53.80 ± 0.01	36.00 ± 0.01	40.80 ± 0.01	40.00 ± 0.10	70.70 ± 0.15	50.00 ± 0.05
		500	56.30 ± 0.02	66.10 ± 0.02	54.20 ± 1.01	58.00 ± 0.02	61.90 ± 0.35	100.00 ± 0.09	81.50 ± 0.07
		1000	67.20 ± 0.02	72.30 ± 0.04	70.00 ± 0.00	100.00 ± 0.08	70.20 ± 0.02	NT	100.00 ± 0.07
2.	Ethanolic Extract	100	33.30 ± 0.07	40.00 ± 0.05	39.10 ± 0.08	47.00 ± 0.07	52.00 ± 0.04	50.00 ± 0.02	31.82 ± 0.01
		250	50.00 ± 0.01	60.20 ± 0.02	66.23 ± 0.10	41.00 ± 0.01	47.00 ± 0.04	66.60 ± 0.18	53.30 ± 0.02
		500	77.70 ± 0.09	98.50 ± 0.08	70.00 ± 0.01	35.00 ± 0.01	40.00 ± 0.09	75.00 ± 0.02	60.00 ± 0.05
		1000	91.10 ± 0.01	100.00 ± 0.01	92.60 ± 0.20	30.00 ± 0.02	36.00 ± 0.18	82.00 ± 0.10	100.00 ± 0.00
3.	Acetone Extract	100	10.20 ± 0.09	16.60 ± 0.15	56.00 ± 0.11	78.00 ± 0.02	25.00 ± 0.16	25.00 ± 0.01	38.00 ± 0.15
		250	40.23 ± 0.04	44.40 ± 0.10	81.80 ± 0.09	86.10 ± 0.06	40.40 ± 0.08	60.00 ± 0.02	51.10 ± 0.10
		500	56.00 ± 0.10	52.00 ± 0.02	100.00 ± 0.02	100.00 ± 0.07	50.61 ± 0.00	100.00 ± 0.10	61.00 ± 0.21
		1000	80.00 ± 0.04	78.50 ± 0.08	NT	NT	100.00 ± 0.20	NT	77.70 ± 0.09
4.	Water Extract	100	38.80 ± 0.07	14.50 ± 0.01	11.10 ± 0.03	17.00 ± 0.07	52.80 ± 0.02	11.68 ± 0.04	27.70 ± 0.11
		250	42.20 ± 0.05	21.80 ± 0.05	24.40 ± 0.05	21.00 ± 0.01	47.50 ± 0.04	20.00 ± 0.08	30.80 ± 0.02
		500	48.80 ± 0.05	32.30 ± 0.05	60.00 ± 0.05	35.00 ± 0.01	47.00 ± 0.04	45.00 ± 0.07	41.10 ± 0.05
		1000	57.70 ± 0.02	40.00 ± 0.07	68.80 ± 0.07	30.00 ± 0.08	36.00 ± 0.05	60.20 ± 0.12	48.70 ± 0.00

potential to discover antimicrobial properties again Bacteria and fungi. The antibacterial activity of methanolic extracts (50 mg/ml) showed the maximum activity against *B. subtilis*. and the acetone extract was the most effective against. *F. oxysporum, C. lunata* and *M. gypsum* which were completely inhibited at 500 mg/ml of concentration.

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REFERENCES :

Agahar-Murugkar, D. and Subbulakshmi, G. 2005. Nutritional value of edible wild mushrooms collected from The Khasi Hills of Meghalaya. *Food Chem.,* **89**: 599-603.

Agarwal GP and Hasuja SK. 1986. Microorgaisms in the laboratory: A laboratory guide of mycology, Microbiology and plant pathology. Print House, Lucknow, India. 155 pp.

Andrew Nyerere, Christine Bii, and Desta Berhe Sbhatu 2019. Determination of Antimicrobial Activity of Extracts of Indigenous Wild Mushrooms against Pathogenic Organisms. Hindawi- *Evidence-Based Complementary and Alternative Medicine. pp.1-7.*

Avnish Kumar, Monika Asthana, Preeti Singh, Meenu Katoch, Prabhu Dutt, Sarika Amdekar, Udita Gubrelay, and Rajendra Sharma. 2020. Antioxidant and antibacterial activity of root extracts of Licorice (*Glycyrrhiza glabra*). *International Journal of Minor Fruits, Medicinal and Aromatic Plants*, **6** (1): 01- 12.

Benedicts, RG. and Brady, L.R 1972. Antimicrobial activity of Mushroom. *J. of Pharma Science,* **61**: 1820-1822.

Chang S. T. and Buswell J. A. 1996. Mushroom nutriceuticals. *World J. Microb. Biotechnol.* **12:** 473- 476. [A summary of the main features of functional foods/nutraceuticals, nutriceuticals and pharmaceuticals].

Chang ST, Miles, PG. 2004. Mushrooms: Cultivation, nutritional value, medicinal effect, and environmental impact, 2nd ed. CRC Press, Boca Raton, FL.

Cristiane U.J.O. Limaa ,Eliana F. Grisb and Margô G.O. Karnikowski.2016. Antimicrobial properties of the mushroom Agaricus blazei – integrative review. *Revista Brasileira de Farmacognosia,* **26:** 780–786.

Daniela Nikolovska-Nedelkoska, N. Atanasova-Pancevska, H. Amedi and Veleska, D. 2013. Screening of antibacterial and antifungal activities of selected Macedonian wild mushrooms. *Journal of Nature and Science*, 124: 333–340.

Grover, R.K. and Moore, J.D. 1962. Toximetric Studies of Fungicides against the Brown Rot Organisms, Sclerotinia fructicola and S. laxa. *Phytopathology*, **52**: 876-879.

Halliwell and Gutteridge, 1984 B. Halliwell, J.M.C. Gutteridge Oxygen radicals, transition metals and disease. *Biochem. J.,* **219**: 1–14.

Jone,s S. and Janardhanan, KK. 2000. Antioxidant and antitumor activity of *Ganoderma lucidum* (curt ex Fr.). P. Karst-Reshi (Aphyllophoromycetieae) from South India. *Int. J. Med. Mushrooms*, **2**: 195-200.

Khedoudja Kanoun, Chama Zouaouia, Zemri Khalida, Harir Noria, Bousmaha Leila and Abbouni Bouziane. 2020. Demonstration of the antifungal activity of the aqueous macerated extract of pomegranate (*Punica granatum* Linn.) bark against Rhodotorula sp. *International Journal of Minor Fruits, Medicinal and Aromatic Plants*, **6** (2): 28-35.

Lakhanpal TN, Rana M. 2005. Medicinal and nutraceutical genetic resources of mushrooms. *Plant Genetic Resources*, **3**(2): 288-303.

Paul Njenga Waithaka1, Eliud Mugu Gathuru, Benson Muriuki Githaiga and Keziah Moraa Onkoba. 2017. Antimicrobial Activity of Mushroom (*Agaricus Bisporus*) and Fungal (*Trametes Gibbosa*) Extracts from Mushrooms and Fungi of Egerton Main Campus, Njoro Kenya. *J Biomedical Sci.,* **6**:3.doi:10.4172/2254-609X.100063.

Smania, A., Dellamonache, F., Smania, E.F.A, Gil, M.L., Benchetrit, L.C. and Cruz, F.S., 1995a. Antibacterial activity of a substance produced by the fungus Pycnoporus sanguineus (Fr.) Murr. *J. Etnno Pharma Col*., **45**: 177-181.

Smania, AJr., , F., Dellmonache Smania, G.F.A. and Cunev, RS. 1997. Antibacterial activity of steroidal compound isolated from *Agaricus bisporus* applanatum (Res.). Pal. (Aphyllopharmyceti deae) fruit body. *Int. J. of Med. Mush.,* **1**: 325-330.

Vincent, J. G. and Vincent, H. W. 1944. Filter paper disc modification of the oxford cup penicillin determination. *Proc. Soc. Exp. Biol. Med.* **55**: 162–164.

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Development of *ex-situ* conservation protocol of Ceylon gooseberry [*Dovyalis hebecarpa* (Gardner) Warb.]

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ABSTRACT

Ex-situ conservation techniques are used effectively to preserve many threatened plant species including Ceylon gooseberry from imminent extinction. The present study was conducted to evaluate an integrated conservation approach which includes seed treatments, seedling establishment and rooting of stem cuttings of Ceylon gooseberry (Dovyalis hebecarpa). Three experiments were set up at the Faculty of Agriculture University of Ruhuna from September 2020 to January 2021. Six seed treatments (seed clipping, cold water soak, hot water treatment, rubbing with fine sand, wood ash, and sandpaper) were used to induce germination of seeds. Germination percentage and germination time were significantly different among treatments. The highest final germination percentage (53%) was recorded when seeds were clipped. The best potting mixture for the seedling growth was observed as topsoil: sand: coir-dust: compost, 1:1:1:2 ratio having 60% seedling survival rate. The commercially available PGR and Aloe vera gel were used to induce rooting in semi-hardwood cuttings. Total root length, number of roots, germination percentage, root and shoot vigour were significantly different among treatments. Ceylon gooseberry seedlings and rooted cuttings could be produced in large scale and establish in the field by adopting the propagation protocol developed in the present study.

Keywords: Ceylon gooseberry, *Ex-situ* conservation, potting mixture, seed treatments, semi-hardwood cuttings

INTRODUCTION

The rapid loss of biodiversity is one of the most critical environmental challenges faced by the present generation. Ceylon gooseberry (*Dovyalis hebecarpa*) is also a threatened plant species that belongs to the family Salicaceae. *Dovyalis hebecarpa* is a perennial shrub growing up to 4-6 meters high with spherical shape berries known as Ceylon gooseberry, or *Ketembilla*. It is well grown in lower mountain rain forest areas in Sri Lanka. Ceylon gooseberry has far-reaching, unique medicinal qualities and it is a source of anthocyanin and other phytochemicals with great potential to be used for drug preparation (Bochi *et al*., 2014). The potential health benefits of phenolic compounds present in Ceylon gooseberry are very important for human health and now investigating the antioxidant functions and disease prevention ability of the fruit (Bochi *et al*., 2015). Ceylon gooseberry fruits contain high antioxidant volume and are acidic fruits. Flavonoids and anthocyanins are the main bioactive compounds responsible for the antioxidant activity of the fruit. In folk medicine, Ceylon gooseberry is often used to medicate infections, eye problems, and diarrhoea. According to Bochi *et al*. (2015), this is a good source of phytochemicals that could be used for the human to provide defence against free radicals. Moreover, the exocarp of the fruit contains higher levels of secondary plant metabolites than the pulp. Hence, exocarp is a promising source of natural pigments and antioxidants for commercial applications. About 40% of plants are now in the threat of extinction. Current literature lacks information about the *ex-situ* conservation methods of this fruit. Therefore the objective of the present study is to develop an integrated approach to *ex-situ* conservation of Ceylon gooseberry.

MATERIALS AND METHODS

The present study comprises three experiments. All experiments were carried out at the Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Sri Lanka from September 2020 to January 2021. Plants were grown under controlled conditions in a protected plant house with average temperature of 40^0C and light intensity of 25,000 lux.

Experiment 1

Effect of different seed treatments on germination

The objective of this experiment was to find out the best seed treatment for rapid and quality germination of Ceylon gooseberry seeds. There were six different mechanical and physical seed treatments, *viz.,* T_1-rubbing with fine sand, T_2-rubbing with wood ash, T_3-rubbing in sandpaper, T_4-cold water treatment, T_5-hot water soak and T_6-seed clipping. A completely randomized design was used to set up the experiment with five replicates. Fully mature fresh fruits were collected from well-grown gooseberry plants in Matara district belongs to the low country wet zone of Sri Lanka. Seeds collection was done in September 2020. The length and width of fruits and seeds were measured using a vernier caliper and fresh weights were measured using an electronic balance. Seeds were extracted from ripening fruits and altogether 150 seeds were randomly selected for applying treatments. Then 25 seeds were subjected to each treatment with five seeds per replicate. For the first treatment river sand with 0.125-0.25 mm particle size was used after sieving by a mesh and rub gently for 10 minutes to remove the slimy coat of the seeds and soften the seed coat. As the second treatment timber wood ash was used and it was also gently rubbed for 10 minutes. For the third treatment, sandpaper was used and rubbed the seed sample carefully for 5 minutes. For cold water soak, seeds were soaked 12 hours in cold water while as hot water treatment, seeds were immersed in a 30^0 C water bath for 30 minutes was practiced. Seed clipping was done by damaging the seed coat slightly using a cutter from the pointed end. Then all seeds were wrapped in wet cotton for 2 weeks and during this period germination status was recorded.

Daily germination was recorded for 14 days. Seeds with about 2mm radical were considered as germinated seeds. The final germination percentage (FGP; Equation 1), germination rate index (GRI; Equation 2), mean germination time (MGT; Equation 3), and mean daily germination (MDG; Equation 4) were calculated using standard equations (Aravind *et al*., 2020; Dharmasena & Arunakumara, 2020) at the end of the experiment.

$$FGP = \frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ seeds\ sown} \times 100 \quad \text{... (1)}$$

$$GRI = N1/T1+N2/T2+N3/T3+....+Nn/Tn \quad \text{..... (2)}$$

N1, N2, N3,……, Nn = number of germinated seeds at a time (days)

T1, T2, T3, …, Tn = number of germinated seeds at a specific time (not the cumulative number).

$$\text{MGT} = \frac{\sum_{i=1}^{k} NiTi}{\sum_{i=1}^{k} Ni} \quad \text{.... (3)}$$

Where; Ti = time taken from the beginning to the i^{th} observation

Ni = number of germinated seeds at the i^{th} time (not the cumulative number, only take the corresponding number relevant to the i^{th} observation)

k = last time of seeds germination

$$\text{MDG} = \frac{Final\ germination\ percentage}{Total\ number\ of\ days} \quad \text{..... (4)}$$

Experiment 2

Effect of different potting mixture for growth and development of seedlings

The objective of this experiment was to select the best potting mixture for seedling growth and survival of Ceylon gooseberry. Germinated seeds of the 1st experiment were sown in nursery trays with compost: sand 1:1 ratio. During the nursery period of three weeks, all the environmental conditions and agronomic practices were equally made to the seedlings to remove any residual effects gained due to various seed treatments. Seedlings which were maintained in nursery trays for three weeks transferred to pots with different potting mixtures (T_1- sand: topsoil: coir-dust: compost 1:1:1:1, T_2- sand: topsoil: coir-dust: compost 1:1:1:2, T_3- sand, topsoil, compost 1:1:1, T_4- sand: coir-dust: compost 1:1:1). Same sized healthy seedlings were selected and planted according to randomized complete block design with five replicates. Survival percentage and a ''vigour'' scale for the seedlings were recorded after 4 weeks. As the ''vigour'' scale, (fully burning -on leaves and wilted nature = 0, partially burning sign-on leaves and partially wilted =1, less than 5 healthy leaves without new buds = 2, less than 5 leaves with new buds = 3, more than 5 leaves with new buds and side branches = 4, more than 5 leaves with new buds, side branches and thorns = 5). Similar visual score was used by Engelbrecht *et al*. (2007) to measure the wilting nature of seedlings due to drought stress.

Experiment 3

Effect of commercially available hormone and *Aloe vera* gel on rooting of semi-hardwood cuttings

The objective of this experiment was to find out the effect of commercially available plant growth regulator (PGR) and the *Aloe vera* gel on rooting of stem cuttings of Ceylon gooseberry. Semi-hardwood cuttings of 4-6 inches in length were established in single propagators of size 16''×5''. All the cuttings were in same length and same number leaves with half removed leaf blade and having few active buds (Ambagaspitiya *et al*., 2020). Five different hormone treatments were used (T_1 - commercial PGR- Rapid root® containing 0.3% Indole 3-butyric acid), T_2 - dipped in *Aloe* gel for 2 minutes, T_3 - dipped in *Aloe* gel for 5 minutes, T_4 - dipped in *Aloe* gel for 10 minutes, and T_5 - without any treatment (control). In the case of *Aloe vera* gel*, Aloe* leaves have been separated from the mother plant two days before extracting gel. The lower portion of the base, the tapering top and the sharp spines of the leaf margin were removed while only the mucilage layer was extracted avoiding vascular bundles, the top rind and the bottom rind (Chandegara and Varshney, 2012). Gel paste was prepared without adding water or any substitute.

General potting mixture of topsoil: sand: coir-dust: compost 1:1:1:1/4 was used for filling single propagators and completely randomized design was applied. Five experimental units with 3 replicates were used for each treatment and data were collected after 2 months of initiating single propagators. Survival percentage (Equation 5), the average length of roots (cm), the average number of roots, roots, and shoot ''vigour'' were evaluated at the end of the trial while lengths of roots were measured manually. ''Vigour'' scales were introduced to measure the roots and shoot development. As root ''vigour'' scale, (no callus formation = 0, callus formation = 1, callus and roots initiation = 2, callus and less than five adventitious roots = 3, callus and more than five adventitious roots = 5) and as shoot ''vigour'' scale, (brown color dried stem = 0, moist stem with a green color = 1, moist stem with green color and less than 3 new buds = 2, moist stem with green color and more than 3 new buds = 3, moist green color stem with more than 3 new buds and less than 2 new leaves = 4, moist green color stem with more than 3 new buds and more than 2 new leaves) were recorded destructively at the end of two months.

$$\text{Survival percentage} = \frac{\text{Number of rooted } \frac{and}{or} \text{ callus formed stem cuttings}}{\text{Total number of cuttings used}} \times 100 \quad \text{..... (5)}$$

Statistical analysis

All the data were analyzed using ANOVA by SAS. Final germination percentage data of experiment 1 and survival percentage data of experiment 3 were subjected to arcsine transformation before analysis. To compare the means of treatments Duncan's Multiple Range Test at 5% probability was used.

RESULTS AND DISCUSSION

Table 1 shows the average fresh weight of fruits and seeds of Ceylon gooseberry. The fruit and seed weight varied in the range of 5-7 g and 55-75 mg, respectively. The average size of fruit was 17.17± 1.83 mm in transversal diameter and 16.72±1.97 mm in longitudinal diameter. Similar fruit characteristics evaluation of *Elaeagnus latifolia* L was done by Rymbai *et al*. (2020). *Dovyalis* fruit color varies during ripening from green to intense brown color and fruits reach their maximum size and biomass accumulation point when the exocarp color is brown while seed removal for propagation should be done when fruits are yellowish-green in hue (Villa *et al*., 2019).

Table 1: Average length, width and fresh weight of fruits and seeds

	Transversal diameter (mm)	Longitudinal diameter (mm)	Weight
Fruit	17.17± 1.83	16.72±1.97	5-7 (g)
Seed	3±1.25	4±1.55	55-75 (mg)

The highest final germination percentage (53%) was recorded in seed clipping treatment (T_6) which is in significant with fine sand rubbing treatment (T_1) (Fig. 1). The lowest germination percentage was observed from hot water treatment (T_5) and sandpaper rubbing (T_3). Duval & Nesmith (2000) reported enhanced seed germination of watermelon after done clipping and removing the seed coat. It was found that pre-soaking for more than 12 hours does not improve germination. Fathurrahman *et al*. (2015) investigated that hot water treatment can reduce germination due to embryo damaging when increase the immersing time for *Albizia saman* plant.

Germination rate index (GRI) defines as the number of seeds germinated within a day and here the highest GRI was recorded from seed clipping (T_6, 0.69) and rubbing in fine-sand treatments (T_1,0.63) (Fig. 2). These two treatments indicate the highest and fastest germination (Table 3). The benefits and weaknesses of GRI have been discussed by Mayer (2000).

The shortest time (5 days) was recorded in seed clipping treatment (T_6) and fine sand rubbing treatment (T_1) while the longest time is taken for germination by seeds treated with hot water (T_5) and sandpaper rubbing (T_3)(8 days) (Table 2). In general, the lowest mean of germination time gives the fastest germination. Hence, the highest germination was recorded in T_6 while the lowest was recorded from T_5. However, T_3, T_5 and T_1, T_6 pairs are not significantly different from others (Fig.1).

The highest mean daily germination (12.80 %) was recorded from seed clipping (T_6) while it was not significantly different with the treatment where seeds were rubbed with fine sand (T_1). The lowest MDG (2.5%) was observed from the hot water treatment (T_5) (Table 2). Similarly, the germination measurements are used to make the interpretations and proper decisions during comparisons. Time, rate and, homogeneity can be measured, depicting the dynamics of the germinations. These characteristics are important to predict the success of a species based on the germination within a time frame. Therefore germination is permitting the recruitment of a plant species in a particular ecosystem (Ranal and De Santana, 2006). According to Hartmann *et al.* (1997), mechanical seed treatments are the best for breaking seed coat dormancy. In the present experiment also gave better result in mechanical and physical seed treatments and the findings was supported from the result of Dahanayake (2015) for 'cardamom seeds'.

In experiment 2, the highest survival percentage and seedling "vigour" were showed by T_2 treatment having sand: topsoil: coir-dust: compost 1:1:1:2 and supporting our results by Khater (2015) who noted the importance of compost for the seedling growth, The lowest survival percentage and seedling vigour were observed from the potting mixture (T_3) prepared with sand, topsoil, compost 1:1:1 and reason may be due to the lack of coir-dust in the mixture and so compaction can be happened (Figure 3A and 3B). Nazari *et al*. (2011) has recommended coir-dust as an efficient substitution for potting mixtures. High total pore spaces and water holding capacity of coir-dust may help to grow seedlings in pots and it has been found that growth index shoot and root dry weight of majesty palm increases in coir-dust media (Meerow, 1995).

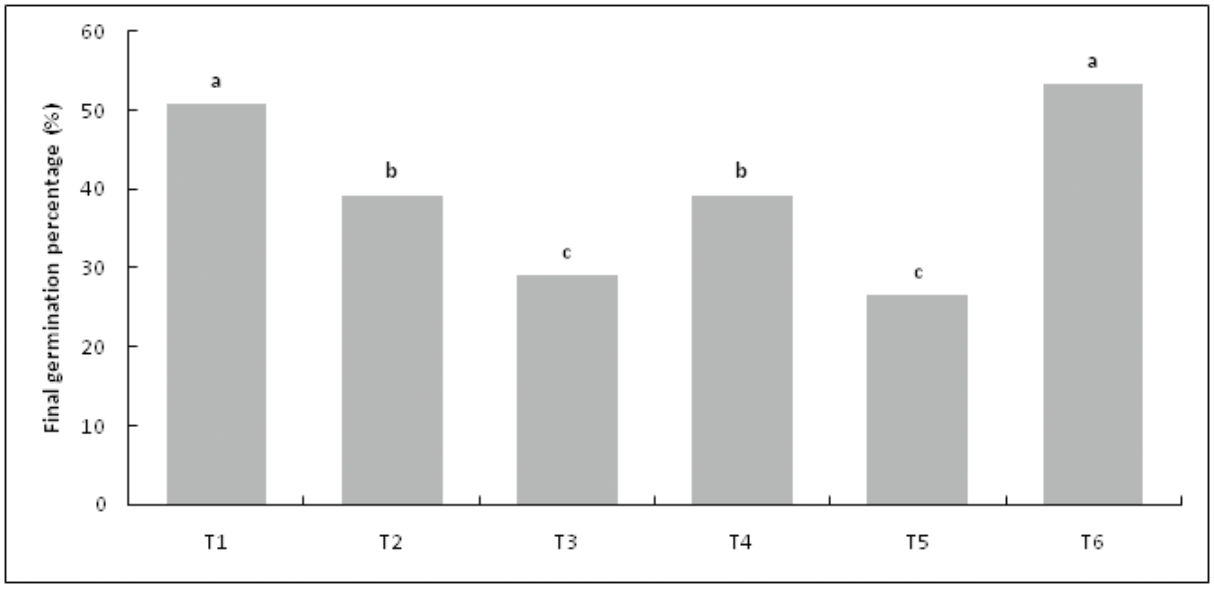

Fig. 1: Effect of different seed treatments on final germination percentage of Ceylon gooseberry seeds. (T_1-rubbing with fine sand, T_2-rubbing with wood ash, T_3-rubbing in sandpaper, T_4-cold water treatment, T_5-hot water soak and T_6-seed clipping)
Means with similar letters are not significantly different from each other in $\alpha = 0.05$)

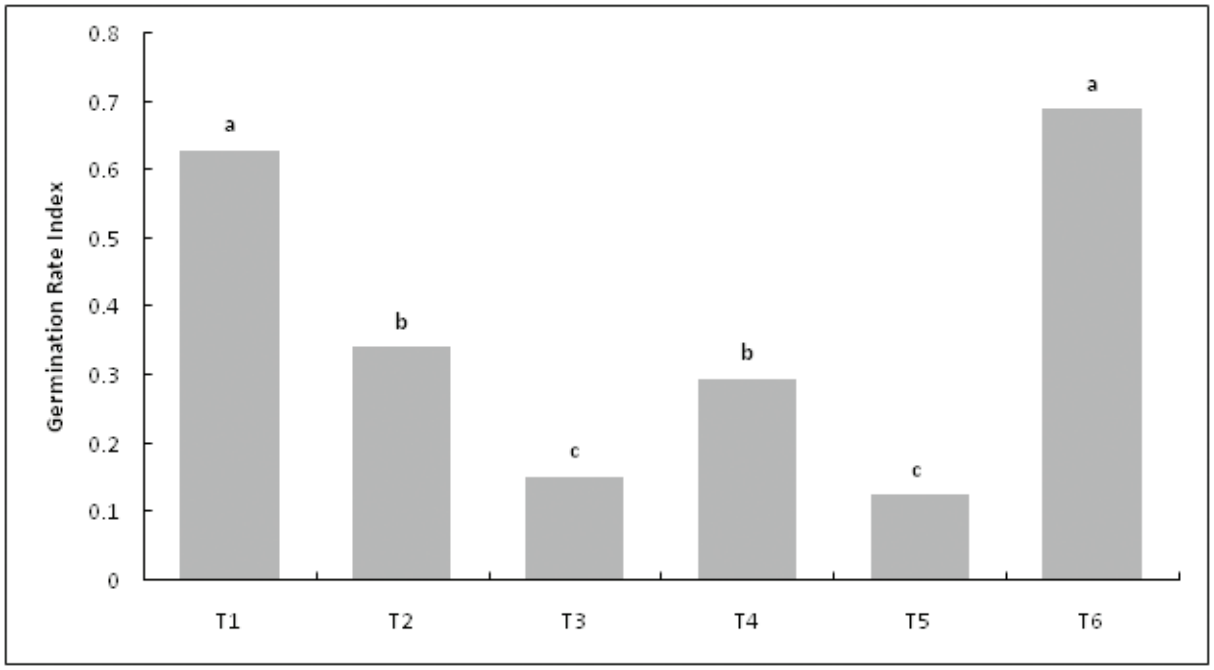

Fig. 2: Effect of different seed treatments on germination rate index of Ceylon gooseberry seeds (T_1-rubbing with fine sand, T_2-rubbing with wood ash, T_3-rubbing in sandpaper, T_4-cold water treatment, T_5-hot water soak and T_6-seed clipping)
Means with similar letters are not significantly different from each other in $\alpha = 0.05$

Table 2: Average time for initiating germination, mean germination time and mean daily germination of Ceylon gooseberry seeds

Treatments	Average time for initiating germination (Days)	Mean germination time	Mean daily germination
T_1	5	4.796^d	12.0^a
T_2	6	5.8^c	6.66^b
T_3	8	7.9^a	3.00^c
T_4	7	6.6^b	5.71^b
T_5	8	8.0^a	2.5^c
T_6	5	4.6^d	12.8^a
CV	21.2	3.197	12.10

(T_1-rubbing with fine sand, T_2-rubbing with wood ash, T_3-rubbing in sandpaper, T_4-cold water treatment, T_5-hot water soak and T_6-seed clipping).
Means with similar letters are not significantly different from each other in $\alpha = 0.05$)

Table 3: Average number of roots, the average length of roots, root vigour, and shoot vigour of semi-hardwood cuttings under different treatments

Treatments	Average number of roots	Average length of roots	Root vigour	Shoot vigour
T1	3.75^a	6.22^a	4.50^a	4.25^a
T2	1.33^c	1.20^c	1.33^c	1.50^c
T3	2.44^b	4.01^b	2.66^b	2.33^b
T4	3.69^a	5.97^a	4.55^a	4.50^a
T5	1.17^c	0.59^d	1.16^c	1.16^c
CV	9.35	8.53	10.10	12.25

(T_1- commercial PGR- Rapid root® containing 0.3% Indole 3-butyric acid, T_2 - dipped in *Aloe* gel for 2 minutes, T_3 - dipped in *Aloe* gel for 5 minutes, T_4 - dipped in *Aloe* gel for 10 minutes, and T_5 - without any treatment -control)
Means with similar letters are not significantly different from each other in $\alpha = 0.05$)

In experiment 3, the commercial IBA hormone treatment (T_1) and dipping in *Aloe vera* gel for 10 minutes (T_4) were not significantly different from each other for the rooting percentage of semi-hardwood cuttings of Ceylon gooseberry which were giving the highest rooting percentage (Figure 4). Control treatment without any hormone application (T_5) was showed the lowest rooting and our findings are fine-tune with the (Almeida *et al*., 2004) who used IBA for air layering of Ceylon gooseberry for getting better success.

The highest average number of roots, length of roots, root, and shoot ''vigour'' were observed from commercial PGR applied cuttings (T_1) and cuttings were dipped in *Aloe vera* gel for 10 minutes (T_4) (Table 3). Increasing in dipping times of stem cuttings in *Alo vera* gel may caused higher accumulation of plant metabolites in the cuttings that increased their rooting numbers and length. According to Mirihagalla and Fernando (2020), root length, the number of roots and root ''vigour'' of the semi-hardwood cuttings of *Citrus aurantifolia* treated commercial PRG (Rapid root®, 0.3% Indole 3-butyric acid; IBA) and dipped cuttings ends in fresh *Aloe vera* gel for two and five minutes were not significantly different. Shidiki *et al*. (2019) showed that IBA, *Aleo vera* gel, and coconut water showed comparable influence on root initiation while number of primary roots was highest in *Aloe vera* gel treatment. They were dipped in 2 minutes in each treatment. Similarly, Uddin *et al*. (2020) showed that the highest root development and survival percentages were recorded from IBA

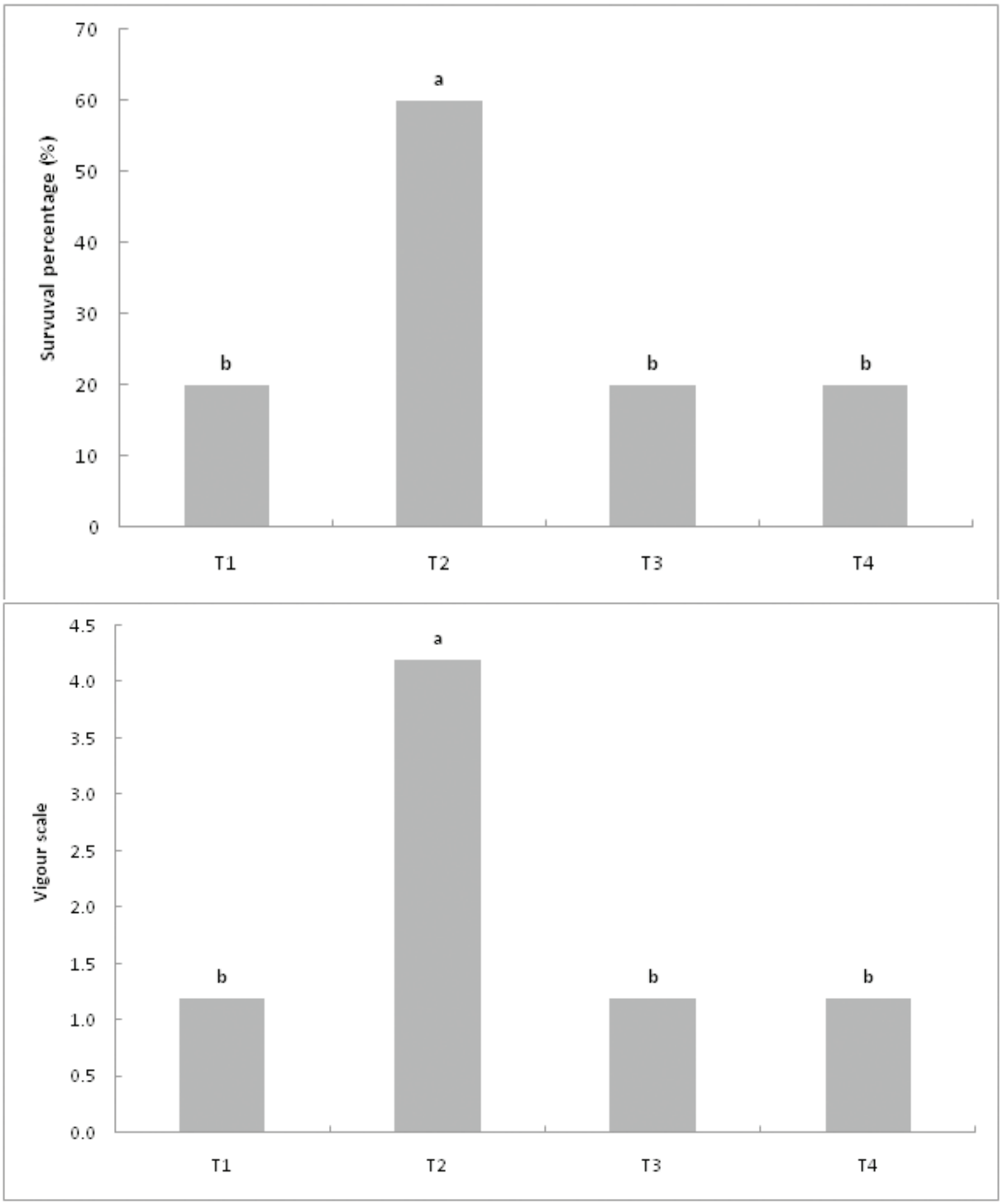

Fig. 3: Effect of different potting mixtures on (A) survival percentage of seedlings and (B) "vigour" of seedlings
(T_1- sand 1: topsoil 1: coir-dust 1: compost 1, T_2- sand 1: topsoil 1: coir-dust 1: compost 2, T_3- sand 1, topsoil 1, compost 1, T_4- sand 1: coir-dust 1: compost 1)
Means with similar letters are not significantly different from each other in $\alpha = 0.05$)

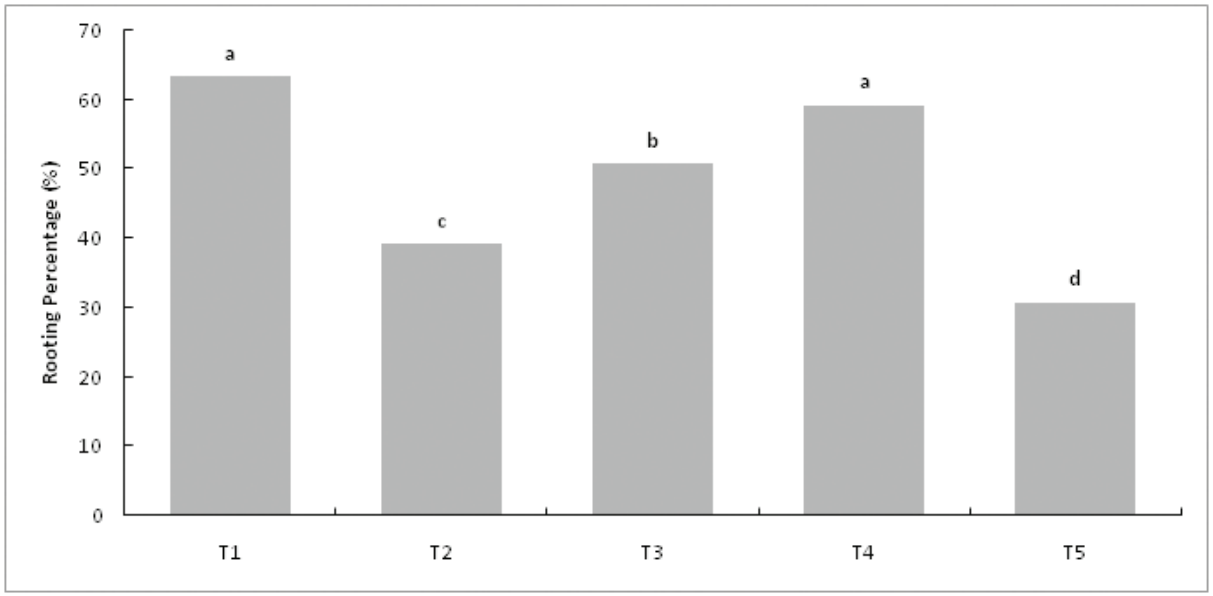

Fig. 4: Effect of different treatments on rooting percentage of semi-hardwood cuttings of Ceylon gooseberry two months after establishment
(T_1- commercial PGR- Rapid root® containing 0.3% Indole 3-butyric acid, T_2 - dipped in *Aloe* gel for 2 minutes, T_3 - dipped in *Aloe* gel for 5 minutes, T_4 - dipped in *Aloe* gel for 10 minutes, and T_5 - without any treatment -control).
Means with similar letters are not significantly different from each other in $\alpha = 0.05$)

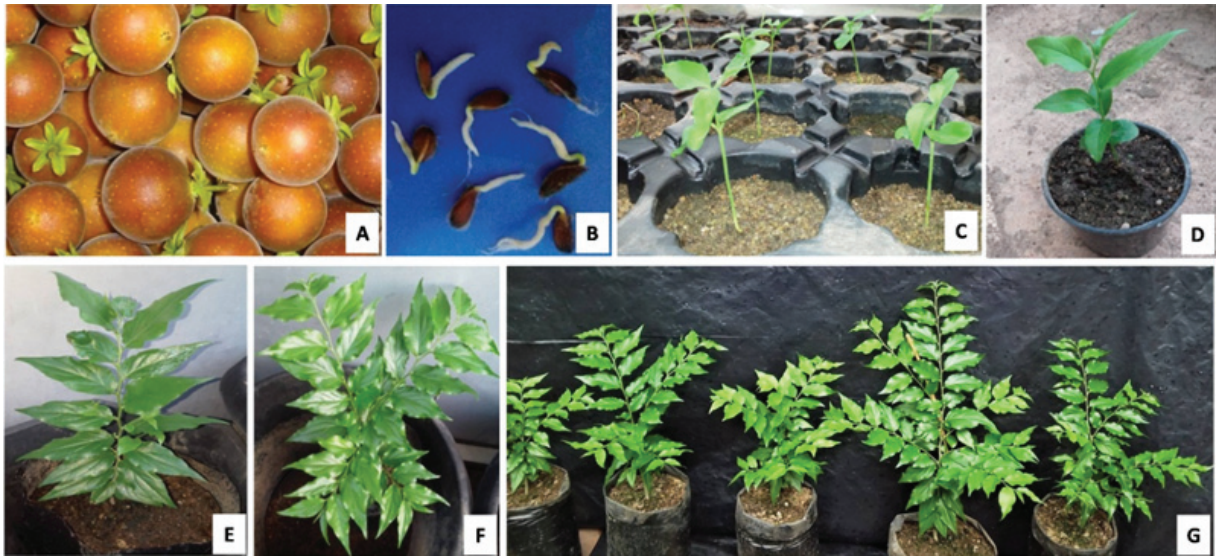

Plate 1: (A) Ripen fruits of Ceylon gooseberry (B) germinated seeds (C) seedling tray nursery (D) transferred seedling to a pot (E) two months old seedling (F) three months old seedling (G) seedlings are ready to be transplanted in the field

which results were closed to natural *Aloe vera* gel treatment. The lowest rooting and "vigour" were showed in the control treatment without any hormone or PGRs (Table 3). *Aloe vera* gel contains mainly polysaccharides and many other compounds like carboxypeptidase, minerals, glucose, vitamins, amino acids, auxins, and gibberellins (Shariff Moghaddasi and Verma, 2011). The use of cuttings for propagation is a fast method and it allows bringing favorable mother characteristics having an agronomic interest (Pourghorban *et al.*, 2019).

CONCLUSION

Conservation of Ceylon gooseberry (*Dovyalis hebecarpa)* species is essential by using propagating techniques since it is gradually disappearing from the natural habitats. According to the results of three experiments, it can be concluded that the physical and mechanical seed treatments can be used to enhance seed germination while potting mixture prepared by sand: topsoil: coir-dust: compost 1:1:1:2 could be used for seedling growth. Commercially available PGRs like Rapid root® (0.3% Indole 3-butyric acid) and cutting ends dipped in *Aloe vera* gel for 10 minutes could be recommended for inducing rooting of semi-hardwood cuttings. Furthermore, for rooting of Ceylon gooseberry, commercial PGRs can be effectively replaced by *Aloe vera* gel. The commercial scale production of Ceylon gooseberry seedlings and rooted cuttings can be achieved by following the propagation protocol developed in the present study.

REFERENCES :

Almeida, E. J. de, Jesus, N. de, Ganga, R. M. D., Benassi, A. C., Scaloppi Junior, E. J., and Martins, A. B. G. 2004. Propagação de Dovyalis sp. peloprocesso de mergulhiaaérea. *RevistaBrasileira de Fruticultura*, **26**(3): 511–514.

Ambagaspitiya, W.U.L., Nawarathna, S.L., Yapa, P.I. and Krishnarajah, S.A. 2020. Propagation of *Bauhinia kockiana* Korth through stem cuttings as affected by maturity stage of cuttings and different biofertilizers. *International Journal of Minor Fruits, Medicinal and Aromatic Plants*, **6**(1):43-49.

Aravind, J., Vimala Devi, S., Radhamani, J., Jacob, S. R. and Srinivasan, K. 2020. The germination metrics package: A brief Introduction. 46. ftp://ftp.onet.pl/pub/mirrors/CRAN/web/packages/germinationmetrics/vignettes/Introduction.pdf

Bochi, V. C., Barcia, M. T., Rodrigues, D. and Godoy, H. T. 2015. Biochemical Characterization of *Dovyalis hebecarpa* Fruits: A source of snthocyanins with high antioxidant capacity. *Journal of Food Science*, **80**(10): C2127–C2133.

Bochi, V. C., Barcia, M. T., Rodrigues, D., Speroni, C. S., Giusti, M. M. and Godoy, H. T. 2014. Polyphenol extraction optimisation from Ceylon gooseberry (*Dovyalis hebecarpa*) pulp. *Food Chemistry*, **164**: 347–354.

Chandegara, V. and Varshney, A.K. 2012. *Aloe vera: Development of gel extraction process for Aloe vera leaves*. LAP LAMBERT Academic Publishing.

Dahanayake, N. 2015. Application of seed treatments to increase germinability of cardamom (*Elettaria cardamomum*) seeds under in vitro conditions. *Sabaragamuwa University Journal*. 14(2): 101. https://doi.org/10.4038/suslj.v14i2.7698

Dharmasena, K. P. S. S. and Arunakumara, K. K. I. U. 2020. Effect of Plant Growth Regulators on Seed Germination in Walla Patta (Gyrinops walla). *Sri Lankan Journal of Agriculture and Ecosystems*, **2**(2): 56-69.

Duval, J. R. and Nesmith, D. S. 2000. Treatment with hydrogen peroxide and seed coat removal or clipping improve germination of "Genesis" triploid watermelon. *Hort. Science,* **35**(1): 85–86.

Engelbrecht, B.M., Tyree, M.T. and Kursar, T.A., 2007. Visual assessment of wilting as a measure of leaf water potential and seedling drought survival. *Journal of Tropical Ecology,* **23**(4): 497-500.

Fathurrahman, Said, M. N. M., Ahmad, W. J. W., Doni, F. and Radziah, C. C. 2015. Germination and seedling response of rain tree plants (*Albizia saman* Jacq. Merr) to seed priming using hot water. Ecology, *Environment and Conservation*, **21**(3): 1183–1187.

Hartmann H. T., Kester D. E., Davies F. Jr. and Geneve R. L. 1997. Plant Propagation Principles and Practices. Sixth Edition. New Jersey, Prentice Hall.

Khater, E. S. G. 2015. Some physical and chemical properties of compost. *International Journal of Waste Resources,* **05**(01). https://doi.org/10.4172/2252-5211.1000172

Mayer, R. F. B. and D. G. 2000. A critical analysis of Maguire's germination rate index. *Journal of Seed Technology,* **10** (2): 101-110.

Meerow, A. W. 1995. Growth of two tropical foliage plants using coir dust as a container medium amendment. *Hort. Technology*, **5**(3): 237–239.

Mirihagalla, M. K. P. N. and Fernando, K. M. C. 2020. Effect of *Aloe vera* gel for Inducing Rooting of Stem Cuttings and Air layering of Plants. *Journal of Dry Zone Agriculture*, **6**(1):13-26.

Nazari, F., Farahmand, F., Khosh-Khui, M. and Salehi, H. 2011. Effects of Different Pot Mixtures on Vegetative, Reproductive and Physiological Characteristics of Iranian Hyacinth (Hyacinthus orientalis L. cv. Sonbol-eIrani). *International Journal of Agricultural and Food Science*, **1**(2): 34-38.

Pourghorban, M., Khaghani, S., Azadi, P., Mirzakhani, A. and Changizi, M. 2019. Propagation of rosa hybrida L. Cv. dolce vita by stenting and stem cutting methods in response to different concentrations of IBA. *Advances in Horticultural Science*, **33**(1): 105–111.

Ranal, M. A. and De Santana, D. G. 2006. How and why to measure the germination process? *Revista Brasileira de Botanica*, **29**(1): 1–11.

Rymbai, H., Deshmukh, N.A., Talang, H.D., Assumi, S.R., Devi, M.B., Mawlein, J. and Jha, A.K., 2020. Evaluation of fruit characteristics of *Elaeagnus latifolia* L. in the North Eastern hill region, India. *International Journal of Minor Fruits, Medicinal and Aromatic Plants*, **6**(1): 38-42.

Shariff Moghaddasi. M. and Verma, S. K. 2011. *Aloe vera* their chemicals composition and applications/ : A review. *International Journal of Biological and Medical Research*, **2**(1): 466-471.

Shidiki, A.A., Ambebe, T.F. and Mendi, A.G. 2019. A comparative evaluation of Indole-3-Butyric Acid and plant extracts as potential rooting enhancers in cuttings of *Vitex diversifolia* and *Cordia milleneii*. *International Journal of Forest, Animal and Fisheries Research*, 3(4):154-159.

Uddin, A.J., Rakibuzzaman, M., Raisa, I., Maliha, M. and Husna, M.A. 2020. Impact of natural substances and synthetic hormone on grapevine cutting. *Journal of Bioscience and Agriculture Research*, **25**(01): 2069-2074.

Villa, F., Fernandes da Silva, D., Rotili, M. C. C., Herzog, N. F. M. and Malavasi, M. M. 2019. Seed physiological quality and harvest point of dovyalis fruits. *Pesquisa Agropecuária Tropical*. V.**49**. e54520.https://doi.org/10.1590/1983-40632019v4954520

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Phenotypical characterization of cultivated eggplants (*Solanum melongena* L.), wild relatives and interspecific hybrids

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ABSTRACT

The study included twenty-two interspecific hybrids of wild relatives of eggplant from primary (S. incanum and S. insanum), secondary (S. lichtensteinii, S. anguivi, and S. dasyphyllum) and tertiary (S. torvum) gene pools, along with cultivated eggplant (Solanum melongena) and their parents. The experiment was laid on completely randomized design and plants were characterized using eggplant descriptor. Out of 18 characters, 12 (growth habit, leaf lobbing, leaf and calyx prickles, anthocyanin distribution, fruit length breadth ratio and color distribution) were significantly different ($p<0.05$) among cultivated eggplants, wild relatives and interspecific hybrids. The total variance in PCA components 1,2 and 3 accounted 50.5%, 20.9%, and 9.9 %, respectively. Three distinguish groups were found in factorial space for the components 1 and 2. Group 1 consisted of MEL1, MEL2, MEL3, MEL4, MEL5, MEL6, MEL8, MEL9 of cultivated eggplants and wild relatives of primary gene pool, INS3, ANG1 and TOR1 in secondary and tertiary genepools respectively. Group 2 accessions highly prickles, with small fruits INS1 from primary and DAS1 from the secondary genepool and interspecific hybrids MEL2×DAS1, MEL7×INS1, MEL2×INS1, MEL5×INS1, MEL6×INS3 associated with this group. Group 3 was made with remaining interspecific hybrids and INS2 from the primary genepool, LIC1 and ANG2 from secondary genepool of wild accessions. It can be concluded dominant alleles related to evaluating morphological characters are carried by the wild relatives. These hybrid materials would be the starting point for introgression breeding in eggplant for climate change they can also be useful as rootstocks against certain biotic and abiotic stress for grafting.

***Keywords**:* Descriptor, genepool, interspecific hybrid, *Solanum melongena* and wild relatives

INTRODUCTION

The eggplant is the most important vegetable crop in the world, in terms of nutritional and medicinal value. In relation with other vegetables, the leaves, fruits and roots have several distinct medicinal values in eggplants, tissue extracts have traditionally been used to treat asthma, bronchitis, cholera and dysuria, fruits and leaves are helpful in reducing cholesterol in the blood (Kashyap *et al*., 2003). Wild relatives of eggplant can grow a wide range of environmental conditions such as deserts with a wide range of temperatures, waterlogged, saline and swampy areas (Davidar *et al*., 2015; Knapp *et al*., 2013 and Lester and Hasan, 1991) besides, resistant to several major diseases (Daunay and Hazra, 2012; Rotino *et al*., 2014). Eggplant wild relatives are grouped, based on genetic relationships and cross ability into different gene pools known as primary, secondary or tertiary (Harlan and Wet, 1971). As *S. incanum* and *S. insanum,* can produce fertile hybrids with cultivated eggplants they are categorized under the primary genepool (Knapp *et al*., 2013). African and Southeast Asian species which resulting hybrids with different levels of fertility are under the secondary genepool (Daunay and Hazra, 2012; Rotino *et al*., 2014). The secondary genepool includes the closely related "eggplant clade", *S. lichtensteinii* and the sister "*anguivi* grade" such as *S. anguivi* and *S. dasyphyllum* (Plazas *et al*., 2016). Tertiary genepool includes species, which result in offspring with sterile or low fertility after embryo rescue or somatic hybridization. Tertiary genepool is an admixture of species from subgenus *Leptostemonum*, which include the old world as well as new world species (Daunay and Hazra, 2012; Rotino *et al*., 2014). Among this genepool, *S. elaeagnifolium*, is an invasive weed with a high ability to stand under drought conditions (Christodoulakis *et al*., 2009) moreover, *S. sisymbriifolium* and *S. torvum* are resistance to multiple diseases (Bletsos *et al*., 2003).

F1 Hybrids of eggplant cultivars that have been growing in greenhouses have a narrow genetic base compared to wild relatives (Mutegi *et al*., 2015; Vorontsova *et al*., 2013). However, wild relatives represent wide variation for resistance traits, which is highly valuable for eggplant breeding (Daunay and Hazra, 2012). Thus, wild relatives play a major role in the preparation of breeding materials having resilient to climate change (Dempewolf *et al*., 2014; Ranaweera *et al*.,2020). Although wild relatives of eggplants have been less utilized in breeding programs, this untapped genetic variation helps to widen the genetic base of eggplant to improve new varieties (Plazas *et al*., 2016). The morphological characterization is a basic step to identifying and effective utilization of wild relatives (Kaushik *et al*., 2016). Thus the experiment was conducted to characterize cultivated eggplants, wild relatives and their interspecific hybrids. Data from wild and cultivated species along with their interspecific hybrids, provide extensive information on the source of diversity, breeding potential and the transfer of key traits from wild relatives to future generations (Prohens *et al*., 2013) also identification potential roots stocks, which can be used as roots stock in grafting which are higher tolerant to biotic and abiotic stresses (Gisbert *et al*., 2011; Daunay and Hazra, 2012).

MATERIALS AND METHODS

The plant materials consisted of 9 accessions of *S melongena* cultivated eggplants originated from Ivory Coast (MEL1, MEL2, and MEL3), Sri Lanka (MEL4, MEL5, MEL6, and MEL9), South East Asia (MEL7) and Spain (MEL8). There are 8 accessions of wild species of primary gene pool *S. insanam* (INS1, INS2, and INS3), Secondary gene pool *S. anguivi*, *S. lichtensteinii*and *S. dasyphyllum* (ANG1, ANG2, LIC1, and DAS1) and Tertiary genepool *S. torvum* (TOR1) (Table 1). 22 interspecific hybrid progenies (Table 2) were obtained by crossing among the aforementioned cultivated eggplants and wild species.

15 plants per parental accessions and hybrids were grown during the *yala* (minor dry) season in 2019 at the experimental station, University of Peradeniya (WM2b). Plants were grown in polythene pots spaced at 60 cm x 90 cm, filled with media having a composition of 5:3:2:1 ratio (topsoil: compost: coir dust: half burn paddy husk) and the experiment was laid according to completely randomized design (CRD). All management practices were done according to the Department of Agriculture (DOA) recommendations. All plants were characterized using an eggplant descriptor ((IBPGR,1990) (Table 3). This descriptor described different traits of the whole plant, leaf, and fruit. Except

Table 1: Accessions of cultivated eggplant (*Solanummelongena*), wild relatives of the primary, secondary and tertiary genepools

Status	Gene pool	Species	Accession	Symbol	Origin
Cultivated		*S. melongena*	MEL1	M1	Ivory Coast
			MEL2	M2	Ivory Coast
			MEL3	M3	Ivory Coast
			MEL4	M4	Sri Lanka
			MEL5	M5	Sri Lanka
			MEL6	M6	Sri Lanka
			MEL7	M7	Commercial (SE Asia)
			MEL8	M8	Commercial (Spain)
			MEL9	M9	Commercial (Sri Lanka)
Wild relatives	Primary	*S. insanum*	INS1	IN1	Sri Lanka
			INS2	IN2	Sri Lanka
			INS3	IN3	Japan
	Secondary	*S. anguivi*	ANG1	AG1	Ivory Coast
			ANG2	AG2	Ivory Coast
		S. lichtensteinii	LIC1	LC1	South Africa
		S. dasyphyllum	DAS1	DS1	Uganda
	Tertiary	*S. torvum*	TOR1	TR1	Unknown (source: France)

Table 2: Interspecific hybrids between cultivated eggplant and wild relatives

	MEL1	MEL2	MEL3	MEL4	MEL5	MEL6	MEL7
INS1		M2×IN1	M3×IN1	M4×IN1	M5×IN1	M6×IN1	M7×IN1
INS2	M1×IN2	M2×IN2	M3×IN2		M5×IN2	M6×IN2	
INS3		M2×IN3	M3×IN3		M5×IN3	M6×IN3	M7×IN3
ANG1							
ANG2		M2×AG2					
LIC1				M4×LC1	M5×LC1	M6×LC1	
DAS1	M1×DS1	M2×DS1					
TOR1							

Table 3: Eggplant descriptor used for characterization

Eggplant descriptors	
Plant growth habit	1- Very upright, 7- Postrate
Leaf-blade lobes	1– Very weak, 9- Very strong
Leaf prickles	0- None, 9- Very many
Leaf length	3- (Short ~ 10 cm), 7- Long ~ 30 cm
Leaf width	3- Narrow ~5 cm, 7- Wide~ 15cm
Leaf tip angle	1-Very acute~ 15^0, 9- Very obtuse~ 160^0
General anthocyanin distribution	0-Absent, 7-Very high
Fruit size	1- Very small <15 g, 9- Very big (>1000g)
Fruit length/breadth ratio	1- Broader than long, 9- Several times longer than broad
Fruit apex shape	3- Protruded, 7- Depressed
Fruit curvature	0- None, 9- U shaped
Fruit shape (position of widest part)	3-About ¼ way from base tip, 7- Above ¾ way from base tip

for plant growth habit, five measurements per plant (per replication) were taken to obtain individual plant average.

Data were analyzed using SPSS software (version 22). Mean values of cultivated eggplants, wild relatives and interspecific hybrid groups were subjected to analysis of variance (ANOVA) and mean separation was done using student-newman-keuls (SNK) at p=0.05. Principal component analyses (PCA) were done using Euclidean distance for each character.

RESULTS AND DISCUSSION

Average values of 12 descriptors were significantly different ($p< 0.05$) among cultivated eggplants, wild relatives and interspecific hybrids out of 18 descriptors. Generally, leaf prickles, fruit calyx prickles, fruit color distribution, anthocyanin distribution of leaf vein and stem and leaf width less in cultivated eggplant compared to wild relatives and interspecific hybrids. Interspecific hybrids had significantly higher leaf prickles and fruit calyx prickles, leaf width, and anthocyanin distribution of stem and leaf veins. Fruit characters including fruit size, number of grooves present in cross-section, and length breadth ratio of wild relatives are significantly lower than cultivated eggplants and interspecific hybrids (Table 4).

The principal component analysis (PCA) performed with agro-morphological descriptors of parental accessions (cultivated and wild relatives) and their interspecific hybrids resulted in main 3 components that explained 81.4% of the total variance. Component 1, 2, and 3 accounted respectively, for 50.5%, 20.9%, and 9.9 % of the total variance.

The first component was negatively correlated with fruit shape (r= -0.618) nevertheless, it was positively correlated with other descriptors such as growth habit (r=0.731), anthocyanin distribution of stem (r=0.759), apex (r=0.721), calyx (r=0.631), leaf blade (r=0.58) and leaf width (r=0.724).The second component was positively correlated with

Table 4: Mean values, standard deviation (SD), and the probability of each morphological character of cultivated eggplant (*S melonogena*), wild relatives, and interspecific hybrids at 0.05 significant level.

Descriptor	Cultivated Eggplant Mean ± SE	Wild relatives Mean ± SE	Interspecific hybrids Mean ± SE	Probability
Growth habit	(5.07±1.48)[b]	(4.72+-1.66)[b]	(3.91+1.32)[a]	0.000
Leaf Lobes	(4.85±1.07)[a]	(5.45+1.50)[ab]	(5.79+1.20)[b]	0.002
Leaf prickles upper	(0±0)[a]	(3.40+4.33)[b]	(2.97+2.57)[b]	0.000
Leaf apex shape	(3.71±0.97)	(4.36+1.29)	(4.10+1.12)	0.113
Leaf length	(4.28±0.97)	(3.63+1.29)	(4.25+1.37)	0.116
Leaf width	(4.96±0.79)[a]	(4.63+1.46)[a]	(5.44+0.83)[b]	0.000
Stem anthocyanin	(1.28±1.84)[a]	(0.95+1.86)[a]	(4.04+2.38)[b]	0.000
Calyx anthocyanin	(0.75±2.20)	(0.40+1.05)	(1.07+1.46)	0.183
Leaf vein anthocyanin	(1.07±1.46)[a]	(0.81±1.36)[a]	(3.44±2.49)[b]	0.000
Leaf blade anthocyanin	(1.07±1.46)	(0.81±1.36)	(3.44±2.49)	0.088
Fruit size	(4.42±1.06)[b]	(2.27±0.98)[a]	(4.08±1.18)[b]	0.000
Fruit length breadth ratio	(5.60±2.88)[b]	(2.54±0.85)[a]	(4.69±1.75)b	0.000
Fruit apex shape	(5.57±1.70)	(6.00±1.60)	(6.18±1.24)	0.126
Fruit color distribution	(3.42±2.63)[a]	(5.09±2.42)[b]	(5.55±2.41)[b]	0.000
Fruit calyx prickles	(0.00± 0.00)[a]	(0.63±1.04)[a]	(2.91±2.75)[b]	0.000
Fruit curvature	(0.64±1.25)[b]	(0±0)[a]	(0±0)[a]	0.000
Fruit shape	(5.42±0.83)	(5.00±0)	(5.14±0.82)	0.114
Fruit cross section	(5.28±2.41)[b]	(3.54±1.96)[a]	(5.00±1.11)[b]	0.000

Table 5: Values represent the correlation coefficients for the three first principal components in the collection of eggplant (*S. melongena*), wild relatives, and interspecific hybrids for each trait. Correlation with absolute values e"0.5 in bold

Trait	Component		
	1	2	3
Growth habit	0.731	-0.428	-0.008
Leaf Lobes	0.293	0.579	0.018
Leaf prickles upper	0.076	0.615	0.005
Leaf apex shape	0.411	-0.177	0.540
Apex anthocyanin	0.721	-0.138	-0.366
Stem anthocyanin	0.759	0.067	-0.113
Calyx anthocyanin	0.631	-0.052	-0.385
Leaf vein anthocyanin	0.818	-0.006	-0.198
Leaf blade anthocyanin	0.580	-0.146	-0.117
Fruit size	0.482	-0.167	0.701
Fruit length breadth ratio	0.323	-0.622	0.352
Fruit apex shape	0.087	0.411	-0.600
Fruit color distribution	0.563	0.236	-0.112
Fruit calyx prickles	0.192	0.566	-0.074
Fruit curvature	-0.142	-0.562	0.244
Fruit shape	-0.618	-0.113	-0.130
Leaf length	-0.019	0.516	0.386
Leaf width	0.724	0.495	0.494
Fruit cross section	0.319	-0.113	0.691

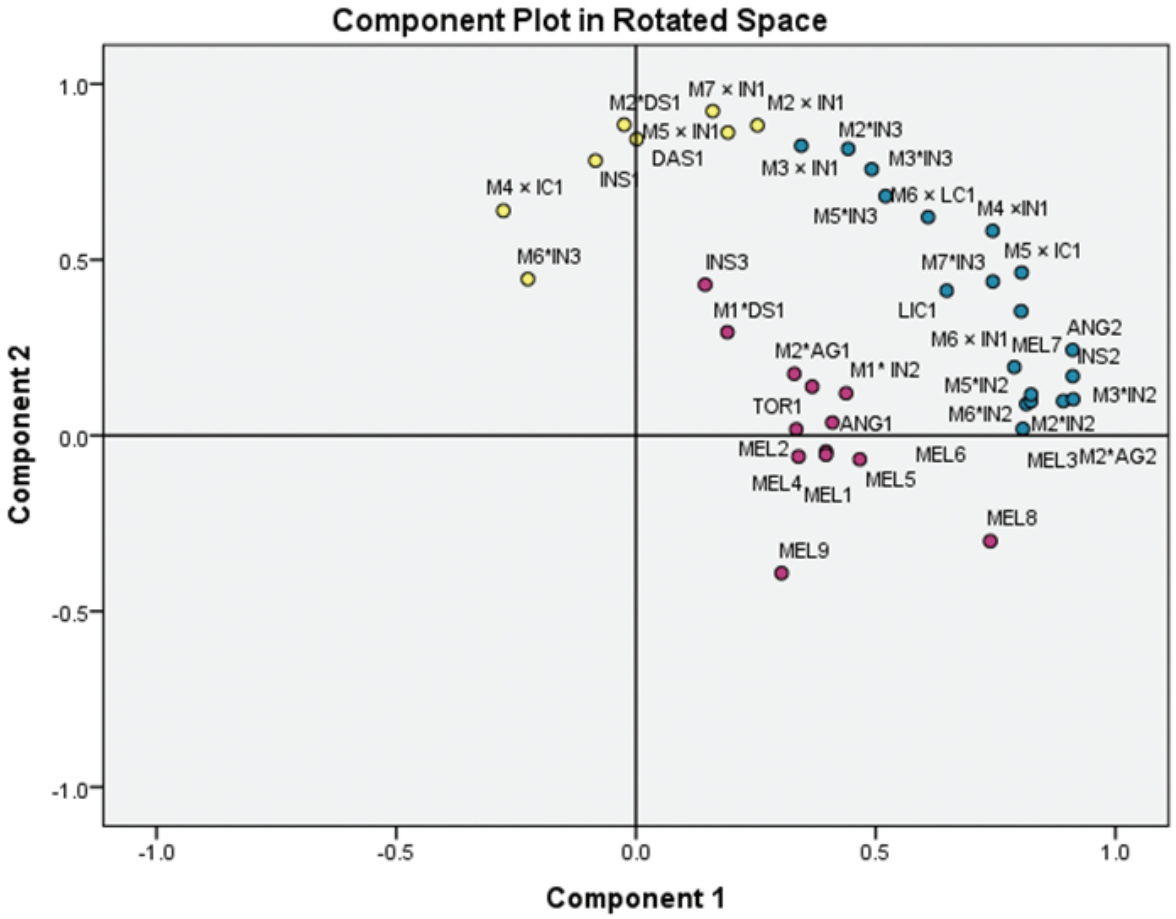

Fig. 1: Distribution of accessions of cultivated eggplant, wild relatives and their interspecific hybrids groups determined by the factorial components 1 and 2 of the PCA

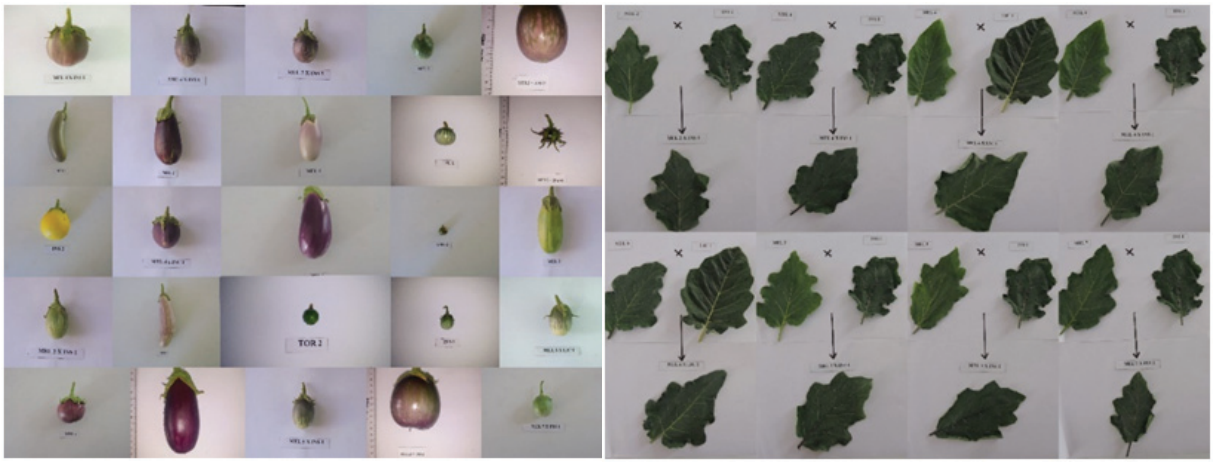

Fig. 2: Fruits and leaves of wild relatives, cultivated eggplants and their interspecific hybrids showing different degrees of morphological characters

leaf lobes (r=0.579), prickles of leaf blade (r=0.615) and fruit calyx (r=0.566) and leaf length (r=0.516) furthermore fruit length breadth ratio (r= 0.622) and fruit curvature (r= 0.562). Leaf apex shape (r=0.540), fruit size(r=0.701) and fruit cross section (r=0.691) was positively correlated with third component and fruit apex shape (r=0.600) negatively correlated (Table 5).

Three distinguished groups were identified in factorial space when drawing a graph with the axes of component 1 and 2 (Fig. 1). Group 1 consisted of MEL1, MEL2, MEL3, MEL4, MEL5, MEL6, MEL8 and MEL9 of cultivated eggplants, and primary gene pool of wild relatives INS3, ANG1, and TOR1 in secondary and tertiary genepools, interspecific hybrids of M1×DS1, M2×AG1 and M1×IN2, also included in this group. This group is with less leaf and fruit calyx prickles, and larger fruit size. Group 2 accessions highly prickles, with small fruits INS1 from primary andDAS1 from the secondary genepool was associated with this group. Interspecific hybrids such as M2×DS1, M7×IN1, M2×IN1, M5×IN1and M6×IN3 also associated with this group. Group3 was made with remain interspecific hybrids and wild relatives of INS2 from the primary genepool, LIC1 and ANG2 form secondary genepool of wild accessions. This represents intermediate characters compared to wild and cultivated accessions.

According to the results, many differences were found among cultivated eggplant, wild relatives and their interspecific hybrids for the morphological traits. Generally, wild varieties can withstand semi-arid and arid environmental conditions (Knapp *et al*., 2013) even though when growing wild relatives and interspecific hybrids under favorable conditions as same cultivated plants, they expressed high vigor than average values for plant morphological features which seems to be an important point to select rootstocks for grafting (Gisbert *et al*., 2011). Reported results showed interspecific hybrids with high prickliness in leaves and fruit calyx, confirming that the prickliness allele was recessive in cultivated eggplants (Doganlar *et al*., 2002; Gramazio *et al*., 2014). Fruit size is a very important trait that is primarily character which consider in breeding programs (Daunay and Hazra, 2012). According to Meyer *et al*. (2012) fruit size varies highly among cultivated eggplants which are becoming prominent with domestication. The fruit size of interspecific hybrids is intermediate among the values of cultivated eggplant and their wild relatives. Even though those values are mostly closer to wild relatives, which confirms wild relatives carrying the responsible genes for fruit characteristics (Doganlar *et al*., 2002). Nevertheless, small fruit can be eliminated by backcrossing in several generations. According to Prohens *et al*. (2013), fruit size can be enhanced by using *S. incanum* even in the first back cross. According to Rotino *et al*. (2014), wild relatives of eggplants used in breeding resulted in undesirable traits such as small fruit size, leaf prickliness, calyx prickliness, etc. As *S. anguivi* and *S. torvum* are with fewer prickles those provide favorable combinations to a breeder. The principal component analysis provides better information regarding the genetic control mechanism of morphological traits. Most of the interspecific hybrids are closer to the wild parent than the cultivated parent. This suggests that the dominant alleles for morphological traits are carrying by their wild parents.

CONCLUSIONS

For the evaluation morphological descriptors of interspecific hybrids are more related to wild parent as dominant alleles related to evaluate morphological characters evaluated in the study are carried by wild relatives. These hybrid materials are the starting point for introgression breeding in eggplant and can be utilized as rootstocks for grafting. Finally, the information reported may have potential value in the development of new cultivars, which are adapted to climate change.

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REFERENCES :

Bletsos, F. Thanassoulopoulos, C. and Roupakias, D. 2003. Effect of grafting on growth, yield, and verticillium wilt of eggplant. *Hort. Scienc*e, **38**(2):183–186.

Christodoulakis, N. S., Lampri, P. N., and Fasseas, C. 2009. Structural and cytochemical investigation of the leaf of silver leaf nightshade (*Solanum elaeagnifolium*), a drought-resistant alien weed of the Greek flora. *Australian Journal of Botany*, **57**(5) 432–438.

Daunay, M. C. and Hazra, P. 2012. "Eggplant," in Handbook of Vegetables, eds K. V. Peter and P. Hazra (Houston, TX: Studium Press), 257–322.

Davidar, P. Snow, A. A. Rajkumar, M. Pasquet, R. Daunay, M. C. and Mutegi, E. 2015. The potential for crop to wild hybridization in eggplant (*Solanum melongena; Solanaceae*) in Southern India. *American Journal of Botany*, **102**(1), 129–139.

Dempewolf, H. Eastwood, R. J. Guarino, L. Khoury, C. K. Müller, J. V. and Toll, J. 2014. Adapting Agriculture to Climate Change: A Global Initiative to Collect, Conserve, and Use Crop Wild Relatives. *Agro ecology and Sustainable Food Systems*, **38**(4), 369–377.

Doganlar, S. Frary, A. Daunay, M.C. and Lester, R.N.T.S. 2002. Conservation of gene function in the *solanaceae* as revealed by comparative mapping of domestication traits in eggplant. *Genetics*, **161**(4), 1713–1726.

Gisbert, C. Prohens, J. Raigón, M. D. Stommel, J. R. and Nuez, F. 2011. Eggplant relatives as sources of variation for developing new rootstocks: Effects of grafting on eggplant yield and fruit apparent quality and composition. *Scientia Horticulturae*, **128**(1), 14–22.

Gramazio, P. Prohens, J. Plazas, M. Andújar, I. Herraiz, F. J. Castillo, E. Knapp, S. Meyer, R. S.and Vilanova, S. 2014. Location of chlorogenic acid biosynthesis pathway and polyphenol oxidase genes in a new interspecific anchored linkage map of eggplant. *BMC Plant Biology*, **14**(1): 350.

Harlan, J. R.and Wet, J. M. J. 1971. Toward a rational classification of cultivated plants. *taxon*, **20**(4), 509–517.

IBPGR 1990. Descriptors for Eggplant. Rome: International Board for Plant Genetic Resources.

Kashyap, V. Vinod Kumar, S. Collonnier, C. Fusari, F. Haicour, R. Rotino, G. L. Sihachakr, D. and Rajam, M. V. 2003. Biotechnology of eggplant. *Scientia Horticulturae*, **97**(1), 1–25.

Kaushik, P. Prohens, J. Vilanova, S. Gramazio, P.and Plazas, M. 2016. Phenotyping of Eggplant Wild Relatives and Interspecific Hybrids with Conventional and Phenomics Descriptors Provides Insight for Their Potential Utilization in Breeding. *Frontiers in Plant Science*, **7**: 677.

Knapp, S. Vorontsova, M. S.and Prohens, J. 2013. Wild Relatives of the Eggplant (*SolanummelongenaL.*: Solanaceae): New Understanding of Species Names in a Complex Group. *Plos One*, **8**(2).

Lester, R. N. and Hasan, S. M. Z. 1991. Origin and domestication of the eggplant, *Solanum melongena*, from *Solanum incanum*, in Africa and Asia. *American Society of Horticulture Science*, **1**, 369–387.

McCouch, S. G. J. Baute, J. Bradeen, P. Bramel, P. K. Bretting, E. Buckler, et al. 2013. Feeding the future. *Nature*, **499(**7456**)**, 23–24.

Meyer, R. S. Karol, K. G. Little, D. P Nee, M. H., and Litt, A. 2012. Phylogeographic relationships among Asian eggplants and new perspectives on eggplant domestication. *Molecular Phylogenetics and Evolution*, **63**(3), 685–701.

Mutegi, E. Snow, A. A. Rajkumar, M. Pasquet, R. Ponniah, H. Daunay, M. C. and Davidar, P. 2015. Genetic diversity and population structure of wild/ weedy eggplant (*Solanum Insanum*, Solanaceae) in southern India: Implications for conservation. *American Journal of Botany*, **102**(1), 140–148.

Plazas, M. Vilanova, S. Gramazio, P. Rodríguez-Burruezo, A. Fita, A. Herraiz, F.J. Ranil, R. Fonseka, R. Niran, L. Fonseka, H. and Kouassi, B.2016. Interspecific hybridization between eggplant and wild relatives from different genepools. *Journal of the American Society for Horticultural Science,* **141**(1), pp.34-44.

Prohens, J., Whitaker, B. D., Plazas, M., Vilanova, S., Hurtado, M., Blasco, M., Gramazio, P.,

and Stommel, J. R. 2013. Genetic diversity in morphological characters and phenolic acids content resulting from an interspecific cross between eggplant, *Solanum melongena*, and its wild ancestor (*S. incanum*). *Annals of Applied Biology*, **162**(2), 242–257.

Ranaweera G.K.M.M.K. Fonseka R.M. and Fonseka H. 2020. Morpho-physiological and yield characteristics of interspecific hybrids between cultivated eggplant (*Solanum melongena L.*) and wild relatives in response to drought stress, *International Journal of Minor Fruits, Medicinal and Aromatic Plants*, **6**(1): 30–37.

Rotino, G. L. Sala, T. and Toppino, L. 2014. "Eggplant," in Alien Gene Transfer in Crop Plants, Vol. 2, eds A. Pratap and J. Kumar (New York, NY: *Springer*), 381–409.

Vorontsova, M. S. Stern, S. Bohs, L. and Knapp, S. 2013. African spiny *solanum* (subgenus l*eptostemonum*, solanaceae): A thorny phylogenetic tangle. *Botanical Journal of the Linnean Society*, **173**(2), 176–193.

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Effect of some nutrients and growth retardant on fruit quality of Wood apple (*Feronia limonia* Swingle)

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ABSTRACT

The experiment on wood apple (Feronia limonia Swingle) conducted at the Horticultural Research Station, Mondouri of Bidhan Chandra Krishi Viswavidyalaya to find out the effect of some nutrients and growth retardant on fruit quality of wood apple. The experiment was laid out in a RCBD with five treatments viz., T_1 - CCC at 0.2 percent, T_2 - KNO_3 at 2.0 percent, T_3 - $ZnSO_4$ at 1.0 percent, T_4 - MAP at 0.8 percent and T_5–control which were replicated four times.The results of the investigation revealed a significant effect of some nutrients and growth retardant on fruit quality of wood apple. Fruit length (7.22 cm), girth (23.17 cm), weight (202.75 g) and pulp contents (67.14 %) were significantly increased in T_1 followed by T_2 (7.21cm, 22.63 cm, 200g and 65.79%), while minimum was observed in T_5 (6.29cm, 19.27cm, 119.25g and 62.50 %). Similarly, the highest TSS (17.35° Brix), and lowest acidity (2.60 %) were found in T_1 followed by T_2 (16.60° Brix, 2.72 %) and the lowest TSS and highest acidity was observed in T_5 (14.95° Brix, 3.10 %). The Vitamin C, Total sugar and reducing sugar were found non- significant. The highest Vitamin C content (4.13mg/100g) was found in T_2 and the lowest in T_5 (3mg/100g). Total sugar 8.28 % and reducing sugar 2.90% were noted in T_1 followed by T_2 (7.72%, 2.81 %) and the lowest in T5 (7.36 %, 2.52%). Therefore, it was concluded that CCC at 0.2 percent and KNO_3 at 2 percentwere found effective for increasing fruit quality of wood apple among the treatments.

Keywords: CCC, fruit quality, KNO_3, mono ammonium phosphate, wood apple, $ZnSO_4$

INTRODUCTION

Wood apple (*Feronia limonia* Swingle; syns. *Limonia acidissima* L.) is a deciduous perennial plant that belongs to the family Rutaceae. In Bengali, it is called katha bel. It is reported to be originated to India. The fruits are eaten as fresh, used for making chutney and jelly. The pectin content of the pulp is 3 to 5% (Krishna *et al*., 2019). Fruit pulp also has many medicinal properties like when unripe, it is used for halting diarrhea, dysentery, high cough, sore throat, the pulp has also anti-inflammatory, antipyretic and analgesic activity. The pulp with honey and pipli (*Piper longum*) is used for curing hiccup and difficulties in breathing (Das, 2018). Leaves and stem bark of *Limonia acidissima* have been practised for anti-tumour and antimicrobial activity (Ahamed *et al*., 2008; Bagul *et al*., 2019).Growth retardant like CCC and nutrients i.e. nitrogen, phosphorus, potassium and zinc play an important role to increase fruit quality of many fruit crops like kinnow mandarin (Gurjar and Rana, 2014), grape (Kumber *et al.*, 2017; Salem *et al.*, 2004) and sapota (Agrawal and Dikshit, 2010). In this view a study was conducted to find out the effect of some nutrients and growth retardant on fruit quality of wood apple.

MATERIALS AND METHODS

The present experiment was conducted at the Horticultural Research Station, Mondouri, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India during the period of 2016- 2018. The soil texture of the experimental field was sandy loam having 6.8 pH. The experimental site was situated in the sub-tropical humid climate where summer and winter both are short and mild. Maximum temperature was ranging from 35.62ºC to 23.35ºC and that of the minimum temperature from 23.75ºC to 8.09ºC during the period of the investigation. Major rainfall was received during the month of June and July. The relative atmosphere humidity prevailed during the period of the experiment varied from 91.32 % to 66.04 %. The experiment was laid out in a Randomized Complete Block Design (RCBD). Which comprised of five treatments viz., T_1 - CCC (cycocel) at 0.2 percent,

T_2 - KNO3 (potassium nitrate) at 2.0 percent, T_3 - ZnSO4 (zinc sulphate) at 1.0 percent, T_4 - MAP (mono ammonium phosphate) at 0.8 percent and T_5 – control and replicated four times. The total number of experimental plants was twenty (10 years old) and plant spacing was 5m. × 5m. Two times spraying was done, first on 10th November 2016 (before bud formation) and second on 10th February 2017 (before flower opening). For physical and chemical analysis of fruits, three fruits for each plant were collected randomly at mature stage. The fruits were then brought to the laboratory and measured. The observations recorded were fruit weight (g), fruit length (cm), fruit girth (cm) and fruit pulp (%). Total soluble solids content (TSS) of fruits estimated with the help of a digital hand refractometer (range 0- 53%) and calibrated at 0° Brix at 20ºC, Total titratable acidity content (%) of the fruits was estimated by titrating the aqueous extract of the known quality of fruit juice against N/10 NaOH alkali solution using phenolphthalein as an indicator and expressed as a percentage (Rusk, 1969). For vitamin C (mg/100g), total sugar (%) and reducing sugar (%) analysis of fruits, the methods were followed as described by A. O. A. C. (1984).

RESULTS AND DISCUSSION

Fruit physical characters i.e. fruit length, girth, weight and pulp content was found significantly improved by foliar application of CCC, KNO_3, $ZnSO_4$ and MAP. The highest fruit length (7.22 cm), girth (23.17 cm), weight (202.75 g) and pulp content (67.14 %) were found with the application of CCC at 0.2% (Table 1) as compare to control (6.29 cm, 19.27 cm, 119.25 g, 62.50 %).The increase in physical characters of fruit due to application of CCC may be attributed to increased accumulation of assimilates and further translocation of extra metabolites through better partitioning towards reproductive growth. The result was supported by Agrawal and Dikshit (2008; 2010), they noted that a significant increase in fruit length, diameter, weight, volume of fruit, pulp thickness, pulp as well as peel weight of fruits of sapota were recorded with the increasing concentration of Cycocel. Similar result was also found by Tripathi and Shukla (2006) in strawberry cv. Chandler when they applied Cycocel (CCC) at 1000 ppm and found highest fruit width.

TSS and acidity of wood apple fruits differed significantly (Table 2) and CCC at 0.2% was found the best in all case with the highest TSS 17.35 ºBrix, and the lowest acidity 2.60 % as compare to control (TSS 14.95º brix and acidity 3.10 %). Similar result was found by Kumber *et al.* (2017) in grape cv. 2A Clone when they applied CCC 750 ppm. In their experiment they found highest total soluble solids (22.01°Brix) and the lowest acidity (0.59 %) as compared to other treatments. In another experiment, Mahalle *et al.* (2010) in acid lime found similar result and they reported that TSS, acidity percentage, improved with the application of cycocel 1000 ppm. The Vitamin C, total and reducing sugar were found non-significant (Table 2), however, highest Vitamin C recorded by application of KNO_3 (4.13 mg/ 100g) as compared to control (3 mg/ 100g). The increased ascorbic acid content with foliar application of potassium might be related with improved sugar metabolism. Gurjar and Rana (2014) also found maximum ascorbic acid (25.81 mg/100 ml juice) in kinnow mandarin fruits with the foliar application of $KNO_3$2%. Total sugar and reducing sugar was found the highest wit the application of CCC (8.28 %, 2.90 %) and the lowest (7.36 %, 2.52 %) in control (Table 2). Cycocel suppresses vegetative growth and therefore a greater chance to utilization and assimilation of total carbohydrates and also might be due to highest TSS content. It was confirmed with the results reported by Kumber *et al*. (2017) in grape cv. 2A Clone. In their experiment they found the highest total sugars (21.50 %), reducing sugar (19.60 %) with application of CCC 750 ppm.

CONCLUSION

Foliar application of CCC, KNO3, ZnSO4 and MAP significantly helped to increased fruit size, fruit weight, pulp contents, TSS and acidity. The heaviest and maximum sizable fruit with high pulp content was recorded from the plants treated with CCC at 0.2%. The highest TSS and acidity of fruits were found with the application of CCC at 0.2%, where, Vitamin C, total sugar and reducing sugar were found non- significant. The highest Vitamin C was noted from the application of KNO_3 at 2%, and highest total and reducing sugar was with the

Table 1: Effect of foliar application of some nutrients and growth retardant on physical parameters of wood apple fruit

Treatments	Length (cm)	Girth (cm)	Weight (g)	Pulp %
CCC @ 0.2 %	7.22	23.17	202.75	67.14
KNO_3 @ 2.0 %	7.21	22.63	200.00	65.79
$ZnSO_4$ @ 1.0 %	6.83	20.79	160.00	63.41
MAP @ 0.8%	6.88	21.26	161.25	64.68
Control	6.29	19.27	119.25	62.50
SE(m) ±	0.198	0.73	18.02	1.01
C.D. at 5%	0.61	2.25	55.53	3.12

Table 2: Effect of foliar application of some nutrients and growth retardanton chemical parameters of wood apple fruit

Treatments	TSS (ºBrix)	Acidity (%)	Vitamin C (mg/100g)	Total sugar (%)	Reducing sugar (%)
CCC @ 0.2 %	17.35	2.60	3.38	8.28	2.90
KNO_3 @ 2.0 %	16.60	2.72	4.13	7.72	2.81
$ZnSO_4$ @ 1.0 %	16.20	2.89	3.19	7.71	2.74
MAP @ 0.8%	16.35	2.78	3.75	7.92	2.86
Control	14.95	3.10	3.00	7.36	2.52
SE(m) ±	0.19	0.08	0.47	0.31	0.09
C.D. at 5%	0.59	0.24	NS	NS	NS

application of CCC at 0.2%. From the results of the investigation it was concluded that two times foliar application *i.e.*, 1st time before flower bud formation and 2nd time before flower opening of CCC at 0.2% or KNO_3 at 2% is helpful for quality fruit production of wood apple.

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REFERENCES :

A.O.A.C. 1984. Official Method of Analysis. *Association of Official Analytical Chemists.* Washington, D.C., U.S.A.

Agrawal, S. and Dikshit, S.N. 2008. Studies on the effect of plant growth regulatorson growth and yield of sapota (*Achrassapota* L.) cv. Cricket Ball. *Indian J. Agric. Res.*, **42**: (3): 207 -211.

Agrawal, S. and Dikshit, S.N. 2010. Studies on the effect of plant growth regulators on qualitative characters of sapota cv. Cricket Ball. *Indian J. Hortic.*, **67**: (2): 177-180.

Ahamed, S.M., Swamy, S.K., Jayaverra, K. N., Rao, J. V. and Kumar, S. 2008. Anti-inflammatory, antipyreticand analgesic activity of methanolic extract of *Feronia limonia*. *Pharmacology,* **3**: 852-857.

Bagul, V., Dhabekar, S., Sansarode, D., Dandekar, S. 2019. Wood Apple (*Limonia Acidissima* L.): A Multipurpose Herb in Cosmetics. *Int. J. Sci. Dev. and Res.*, **4**: (7):172-181.

Das, A. 2018. Ethnobotanical uses of wild fruits of Santal paraganas (Jharkhand). . *Intl. J. Minor Fruits Med. Arom. Plants*, **4** (2): 31-38.

Gurjar, P.S. and Rana, G.S. 2014. Inûuence of foliar application of nutrients and growth regulator on fruit drop, yield and fruit size and quality in Kinnow mandarin. *Indian J. Hort.,***71**: (1):109-111.

Krishna, H., Saroj, P.L., Maheshwari, S.K., Singh, R.S., Meena, R.K., Chandra, R. and

Parashar, A. 2019. Underutilized fruits of arid & semi-arid regions for nutritional and livelihood security. *Intl. J. Minor Fruits Med. Arom. Plants*, **5** (2): 01- 14.

Kumbar, S., Patil, D.R., Das, K.K., Swamy, G.S.K., Thammaiah, N., Jayappa, J. and Gandolkar, K. 2017. Studies on the Influence of Growth Regulators and Chemicals on the Quality Parameters of Grape cv. 2A Clone**.** *Int. J. Curr. Microbiol. Appl. Sci.,* **6**: (5)**:** 2585-2592.

Mahalle, S.S., Ingle, H.V. and Sable, P. B. 2010. Influence of plant growth regulators and chemicals on yield and quality of *Hasta bahar* in acid lime. *Green Farming*, **1**: (3): 285-287.

Rusk, J. A. 1969. Chemical methods for analysis of fruit and vegetables product, Research Station, Summerland, B.C. Canada Deptt. of Agriculture.

Salem, A. T., Kilany, A. E.and Shaker, G. S. 2004. The influence of NPK, phosphorus source and potassium foliar application on growth and fruit quality of Thompson Seedless grapevines. *Acta Hortic*., **640:** 163-173.

Tripathi, V.K. and Shukla, P.K. 2006. Effect of plant bio-regulators on growth, yield and quality of strawberry cv. Chandler. *J. Asian Hort.,* **2**(4): 260-263.

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Morphological characterization of *Terminalia chebula* (Aralu) and their propagation techniques

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ABSTRACT

Terminalia chebula (Aralu) is a valuable medicinal plant, which has an amazing power to combat various kinds of ailments.The plant becomes endangered due to its poor natural resurgence, unsustainable harvesting together with over exploitation. The conventional propagation of Terminalia chebula is through seeds and their germination is very low.This study was focused on morphological characterization of Terminalia chebula germplasm and to select suitable vegetative propagation method. A field survey was carried out in Galle and Matara districts, in Southern province of Sri Lanka for germplasm characterization. Ten plants were selected from each district. Information on growing environment, plant, leaf and fruit characters were collected and compared with the herbarium of Royal Botanical Garden. Data were subjected to a cluster analysis and two sample t test. In subsequent experiments, grafting, budding and air layering were practiced and they were carried out at the medicinal plant garden, Pinnaduwa, Galle. One-year old healthy rootstocks of Terminalia catappa (kottamba) and Terminalia bellerica (bulu) were used for wedge grafting and patch budding while keeping Terminalia chebula as the scion. Completely randomized design in factorial arrangement was used with 9 replicates. Air layering was carried out using two hormone types together with their three different concentrations including 500,700 and 900 ppm. Randomized complete block design was used with three replicates. Six different categories of Terminalia chebula were identified by cluster analysis. Both Terminalia catappa and Terminalia bellerica rootstocks can be used for vegetative propagation of Aralu. The highest survival percentages of 78% and 67% were recorded respectively in wedge grafted Terminalia chebula using Terminalia catappa root stock and patch budding with Terminallia bellerica. Types and concentrations of hormones used were not effective for Terminalia chebula air layering. Different categories of Aralu reported in study provide clues for its existing considerable genetic variability. Chemical compounds present in these six different categories warrant further investigation.

***Keywords**:* Endanger, morphological characters, seed germination, vegetative propagation

INTRODUCTION

Aralu (*Terminalaia chebula*) is a moderate deciduous tree belongs to the family combretaceae. It is a multipurpose medicinal agroforestry tree species and a popular herbal remedy in India and South East Asia. All the parts of the tree are used in traditional unani and homeopathic medicines for treating different ailments. The dried fruit is the most prominent part used in healthcare practices. *Aralu* is used as an appetizer, laxative agent, used to cure nervous weakness (Surya Prakash *et al*., 2012). It is used as a homemade remedy for asthma, sore throat, vomiting, hiccough, diarrhea, bleeding piles and ulcers (Anwesa *et al*., 2013).The tree is usually propagated through seeds that are hard and pale yellow. However, long time taken for germination and poor germination percentage exist as major bottlenecks in seed propagation (Hossain *et al.,* 2005). Vegetative propagation is possible through stem cuttings and by grafting (Jose and Thomas,1998). *Aralu* tree is naturally distributed only in the intermediate zone in Sri Lanka. But planted trees can be seen in all other agro- climatic zones in the country (Sanjeewa *et al.,*2015). However this species has received a little attention by the researchers in Sri Lanka.There is no emphasis given in varietal identification and selection of plus trees for its improvement and commercial cultivation. Therefore, it is important to ascertain genetic or phenotypic variations of available *Terminalia chebula* populations in different regions in Sri Lanka. In order to increase

the production and population of the tree, the total land area under cultivation and productivity should be increased. For this expansion and improvement, it is necessary to implement well-planned research, conservation strategies and vegetative propagation techniques to produce early bearing, high yielding plants. Morphological characterization of elite Jamun (*Syzigium cuminii* Skeels) has been practiced in India (Swamy *et al*., 2017), which can be used in identification of genotypes, selection and crop improvement programs. Therefore, this study was undertaken for identification of elite genotypes and standardization of vegetative propagation techniques.

MATERIALS AND METHODS

The research was conducted as two experiments. The experiment on "morphological characterization and identification of *Terminalia chebula* germplasm" was conducted as a field survey in Galle and Matara districts. These two districts were selected for the study because considerable extents of *Terminalia chebula* plants were recorded to have in these two regions. Ten plants were selected from each district. A structured questionnaire was used to gather information. Environmental characters (site topography, associated vegetation, status of sample), plant characters (height, age, trunk circumference, tree shape, growth habit, branching density, bark color), leaf characters (leaf blade length, blade width, blade shape, apex shape, blade margin, venation, petiole length), fruit characters (bearing habit, fruit shape, apex and base shape, number of ridges on fruit, diameter of fruit, fruit and seed weight) were measured during this survey. Varietal identification was done by referring to the herbarium of royal botanical garden. A cluster analysis and a two sample t test was carried out by considering all the observations (Minitab version 16). The experiment on "selection of suitable vegetative propagation method" was conducted in medicinal plant garden, Pinnaduwa, Galle. Wedge grafting, patch budding and air layering were practiced. One year old healthy vigorous plants of *Terminalia catappa* and *Terminalia bellerica* were used as root stocks for budding and grafting.Wedge grafting and patch budding were arranged in a factorial completely randomized design with nine replicates per each. Altogether there were 36 experimental units. Success percentages of grafted unions were observed after 28 days of grafting. Collected data on survival was analyzed using ANOVA in SAS software and treatment means were compared using DMRT. Air layering was practiced using three selected ten years old Aralu trees. Healthy, vigorous, semi hardwood, lateral and pencil thickened: about 30-40cm length shoots of the trees were selected for layering. Two different types of hormones were used, as pure Indole Buteric Acid (IBA) and commercial hormone rootone (0.03% IBA). Three concentrations of each two hormones were used as 500ppm, 700ppm, 900ppm, and a control treatment was practiced without using hormones. Altogether seven treatments per each tree were carried out in a randomized complete block design with three replicates. The bark of the shoots was removed as a ring to a 1 to 2 cm length. The rooting hormones were applied to the cutting surface and the treated area was wrapped with transparent 300 gauge polythene. Moist and sterilized coir dust was used as the rooting medium. The percentages of survival were taken in each two weeks interval period up to eight weeks.

RESULTS AND DISCUSSION

According to the results of the survey, almost all the samples studied were planted trees. No naturally occurring trees were observed in Galle and Matara districts. According to Sanjeewa *et al*. (2015) the *T. chebula* trees are naturally distributed only in the intermediate zone of Sri Lanka, although planted trees can be seen in all agro-climatic zones. All the tree samples studied were surrounded by other vegetation types such as *Cinnamomum verum, Mangifera indica, Alstonias cholaris, Mesuaferrea, Anacardium occidentale, Cocos nucifera, Gmelina arborea, Artocarpush eterophyllus, Terminalia catappa, Caryotaurens, Syzygium samarangense, Artocarpus nobilis, and Musa spp etc*. The tree shapes were varied as round, spread, open, irregular and oval. Sixty percent of the samples showed a vigorous growth habit, while 20% exhibited high and less growth habits. Majority of the tree samples exhibited higher branching density (45%) while 30% moderate and 25% low branching densities respectively. The results of some other tree characteristics are represented in the table 1. Genotype M6 is the older Aralu tree which is 130

Table 1: Tree characteristics of *Aralu* identified in Galle and Matara districts

District	Genotype	Tree height (ft)	Diameter at base (cm)	Diameter at breast height (cm)	Girth at base (cm)	Girth at breast height (cm)
Galle	G1	50.2	84.0	53.5	264.0	168.0
	G2	20.6	53.0	39.8	166.5	125.0
	G3	30.0	46.0	37.2	144.5	117.0
	G4	55.5	74.5	36.6	234.2	115.0
	G5	25.1	22.5	16.1	70.7	50.5
	G6	18.5	15.0	9.8	47.1	31.0
	G7	23.0	24.0	13.5	75.4	42.5
	G8	30.8	12.3	10.5	38.6	33.0
	G9	10.0	8.2	4.4	25.9	14.0
	G10	8.2	6.5	4.1	20.4	13.0
Matara	M1	30.5	55.4	30.9	174.2	97.0
	M2	45.3	86.0	64.9	270.2	204.0
	M3	50.2	79.6	68.4	250.2	215.0
	M4	55.0	67.5	45.8	212.1	144.0
	M5	60.8	78.2	53.5	254.9	168.0
	M6	50.1	91.0	73.2	286.0	230.0
	M7	40.5	89.8	71.6	282.3	225.0
	M8	25.0	29.2	25.8	91.9	81.0
	M9	24.0	24.3	25.7	91.5	82
	M10	45.0	85.2	74.8	210.1	145.0
P value		**0.001**	**0.000**	**0.021**	**0.003**	**0.000**
SD (Standard Deviation)		**13.34**	**24.85**	**68.61**	**92.31**	**57.16**

Note : Measured tree characteristics for 10 plants per each district with p values and SD of two sample t test significant at 0.05 level

years old. The tree exhibits the highest diameter at base (91cm), diameter breast height (73.2cm), higher girth at base (286cm) and highest girth at breast height (230cm). According to the two sample t- test results, the height of the trees (p=0.01), the diameter at base (p= 0.000), girth at base (p=0.003), diameter breast height (p= 0.021), girth at breast height (p=0.000) of the trees were significantly different (p=0.05) from one region to the other. Based on a research conducted in different agro ecological zones in Sri Lanka, it has revealed that variations were observed in stem/trunk characters, leaf characters and fruit characters of *T. Chebula* (Sanjeewa *et al*., 2015).

According to the two sample t test carried out for the leaf characteristics of the *Aralu* tree, the average leaf blade length (p= 0.1), average leaf blade width (p= 0.4), average petiole length (p=0.4) of Galle and Matara districts were not significantly different. However, both between-and within-tree variations in different agro-ecological zones of Sri Lanka were significant (p<0.01) in terms of leaf characters (Sanjeewa *et al*., 2015).The highest average leaf blade length (13.4cm) was recorded from *Aralu* tree at Nagaramaya temple in Galle. The lowest leaf blade length (7.4cm) was observed from a tree at Pinnaduwa medicinal plant garden. Most of the plants in two districts have leaf blade lengths more than 8 cm. The highest average leaf blade width was 8.4 cm and lowest leaf blade width of 4.1 cm was recorded from Nagaramaya temple and Pinnaduwa medicinal plant garden respectively. The highest leaf petiole length (3.2 cm) and the lowest (0.9 cm) reported at Nagaramaya temple and Pinnaduwa medicinal plant garden respectively. Figure 1 represents the variation of leaves in different *Aralu* trees in Matara district.

Table 2: Fruit characteristics of Aralu fruits collected from Galle and Matara districts

Genotype/ Fruit parameter	G1	G2	G3	G4	M1	M2	M4	M5	P value	SD value
Fruit shape	Triangular	Elongated	Round	Elliptic	Elliptic	Elliptic	Elliptic	Elliptic		
Fruit apex shape	Attenuated	Obtuse	Round	Obtuse	Obtuse	Obtuse	Obtuse	Obtuse		
Number of ridges	5	7	7	5	5	6	6	5	0.441	0.8754
Fruit diameter (cm)	2.9	1.8	2.4	2.6	1.6	2.5	2.5	1.8	0.032	0.4631
Fruit length (cm)	3.7	4.0	3.4	3.7	3.4	2.8	2.8	2.5	0.000	0.2739
Fruit weight (g)	9.6	6.8	8.4	10.4	9.6	4.2	4.2	3.6	0.000	1.0246
Fruit dry weight (g)	6.6	3.9	4.7	7.3	6.7	3.1	3.1	2.5	0.000	1.2577
Seed weight (g)	5.5	3.2	4.4	6.2	5.6	2.8	2.8	2.2	0.000	1.0165
Moisture %	31.6	42.0	43.73	29.5	29.9	25	25	30.1	0.000	4.0424

Measured fruit characteristics with p values and SD of two sample t test significant at 0.05 level

Table 3: Identified categories and genotypes

Category	Genotypes
A	G1, M5
B	G2, G3, M1
C	G4, M4
D	G5, G7, M8
E	G6, G8, G9, G10, M9, M10
F	M2, M3, M6, M7

(G- Galle, M- Matara)

Fruit characteristics observed in the samples from Galle and Matara districts are represented in the table 2. There were four types of fruit shapes observed as triangular, elongate, round and elliptic. Almost all the fruit samples in Matara district were elliptic in shapes were attenuated, obtuse and round. Fruit apex shape of all the samples in Matara district were obtuse. According to the two sample t test carried out, number of ridges on the fruits (p=0.441) was not significantly different in both regions. But average fruit diameter (p=0.032), average fruit length (p=0.000), average fresh fruit weight (p= 0.000), average dry fruit weight (p=0.000), average seed weight (p=0.000), average moisture % (p=0.000) in Galle and Matara districts were significantly different. According to Sanjeewa *et al*. (2013), the within tree variation for fruit characters was not significant (p>0.01), while the between trees was significant (p<0.01) for fruit characters such as fruit shape and size. Six Aralu varieties in Galle and Matara districts were identified by the cluster analysis. Genetic variability is key prerequisite in any breeding programmes for tree improvement (Navhale *et al*., 2011). The category and the genotypes included in each category is represented in the table 3. In the royal botanical garden, no any data were available for varietal identification of this species.

Results of the experiment of budding and grafting of *T. chebula* using different rootstocks are represented in the table 4. Significantly highest survival percentage of *T.chebula* (77.7%) was recorded in wedge grafted plants using *Terminalia catappa* root stocks. And patch budding with *Terminalia bellerica* rootstock was successful at a rate of 66.6%. But air layering of *Aralu* by using different concentrations of IBA and commercial hormone did not form roots. Only a callus formation on the layered surface was observed after eight weeks period.

Fig. 1: **Leaf variation of different trees at Matara district**

Table 4: Survival percentage of *Terminalia chebula* at four weeks after budding and grafting

Treatment	PropagationType	Rootstock	Scion	Survival %
TCG (*T.catappa* Grafting)	Wedge grafting	*Terminalia catappa*	*Terminalia chebula*	77.7[a]
TCB (*T.catappa* Budding)	Patch budding	*Terminalia catappa*	*Terminalia chebula*	44.4[c]
TBG (*T.bellerica* Grafting)	Wedge grafting	*Terminalia bellerica*	*Terminalia chebula*	33.3[d]
TBB (*T.bellerica* Budding)	Patch budding	*Terminalia bellerica*	*Terminalia chebula*	66.6[b]

Means followed by the same superscripts are not significantly different at p>0.05

CONCLUSIONS

From this present investigation, it is concluded that six categories of *T. chebula* were identified and this provide a clue for wide genetic variability of *Terminalia chebula*. For vegetative propagation of *Terminalia chebula, rootctocks of Terminalia catappa* and *Terminalia bellerica* were found to be suitable. Further, propagation through air layering without or with the concentrations of 500 ppm, 700 ppm, 900 ppm of IBA and commercial hormone are not much effective in air layering of *Aralu*.

REFERENCES :

Anwesa, B., Subir, B.K. and Rabi, C.R. 2013. Therapeutic potential of *Terminalia chebula* Retz.(Combretaceae): the ayurvedic wonder, *Asian Pacific Journal of Tropical Biomedicine*, **3**: 244-252.

Hossain, M.A., Arefin, M.K., Khan, B.M. and Rahman, M.A. 2005. Effects of seed treatments on germination and seedling growth attributes of Horitaki (*Terminalia chebula* Retz.) in the nursery. *Research Journal of Agriculture and Biological Sciences,* **1**(2): 135-141.

Jose, P.A and Thomas, J. 1998. An account of the vegetative propagation in *Terminalia chebula* Retz, *The Indian Forester,* **124** (5):357-359.

Navhale, V.C, Sonone, N.G., Jangam, P.S., Jadhav, S.T and Bhave, S.G. 2011. Genetic variability and selection of candidate plus trees in chebulic myrobalan (*Terminalia chebula* Retz.). *Electronic Journal of Plant Breeding*, **2**(1): 157-163.

Sanjeewa, T.A.B.D., Pushpakumara, D.K.N.G. and Sangakkara, U.R. 2013. Morphological characterization of *Terminalia chebula* Retz. in Sri Lanka, *Tropical Agricultural Research,* **25**(1): 127-132.

SuryaPrakash, D.V., Sreesatya, N., Avanigadda, S. and Vangalapati, M. 2012. Pharmacological review on *Terminalia chebula*. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, **3**(2) : 679-83.

Swamy, G.S.K., Anushma P.L and Jagadeesha R.C. 2017. Morphological characterization of elite Jamun (*Syzigium cuminii* Skeels) genotypes, *International Journal of Minor Fruits, Medicinal and Aromatic Plants*, 3 (1): 09-15.

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SHORT COMMUNICATION

Ethno-medicines used by Santals & Paharias for treating skin diseases

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ABSTRACT

Ethno-medicines are being practiced by the local herbal healers known as Pahans, Horopaths, Manjhis, Nayakis and Vidyas of Dumka district in the state Jharkhand, India. These medicinal practitioners are treating number of diseases and disorders including the skin problems using available ethno-medicines of the surrounding forest and hilly areas of the region. The main objective of this paper is to explore and enumerate these highly significant 55 ethno-medicinal plant species used by these people in skin treatment, along with their local names, families, parts used and ethno-medicinal uses. These ethno-medicines may further provide future scope to nutrition, escort bio-molecules for the development of new drugs, further experimentations, explorations and researches for various economic aspects, profiting our society.

Keywords **:** Ethno-medicines, explorations, traditional-knowledge

INTRODUCTION

Tribal populations of Dumka district, mainly the *Santhals* and *Paharias* and some indigenous communities like *Bhumijs, Mahalis, Kols, Napits* and *Kumhars* reside, in the lap of extensive forests, several scattered hillocks, high ridges, valleys and beside rivers. These peoples specially the herbal healers are treating a number of ailments and diseases including the skin diseases by the available ethno-medicines, which they are practicing over generations and have learned verbally, from their ancestors. Ironically, this vital knowledge is inherited verbally, from elders of the society to youngsters, without any basic documentation, experimental assessment and inventory preparations. And along with the gradual modernization, the lives of these communities are getting tough and hence are either migrating to other areas or changing their profession, ultimately, this knowledge is threatened. Hence there is an urgent need to conserve and sustain this traditional knowledge along with these tribal and indigenous people who know pretty well to utilize the bio-resources sustainably without disturbing the ecology.

This paper explores and enumerates 55 such ethno-medicinal plant species being utilized by these communities in skin treatment. These further provide scopes for explorations of various economically important plant species, which are being utilized by these people to meet their primary health and daily requirements.

MATERIALS AND METHODS

Thorough and extensive field work was conducted in different randomly selected blocks of the district namely, Kathikund, Shikaripara, Gopikandar, Rangamission, Dumka, Maharo and Jama, since 2018-2020 especially in the months of December to August, to collect most of the species in their flowering and fruiting season. For this very purpose, semi-structured questionnaires were prepared. Ethnic and knowledgeable, herbal medicinal practitioners were interviewed several times. They were interviewed for the type of plants and preparation of the drugs and their doses; they use to cure the patients. Separate interviews were also made with the patients for knowing the degree of cure.

A total of fifty five ethno-medicinal plant species were collected which are being utilized in skin treatment, properly tagged with their local names. Collected plants were processed and herbarium specimens were prepared following standard herbarium techniques (Jain and Rao, 1977) and identified consulting available literatures (Haines, 1921-1925; Kirtikar and Basu, 1935; Anonymous, 1948-1976; Chopra *et al.*1956; Maheshwari and Singh, 1965 and Jain, 1968).

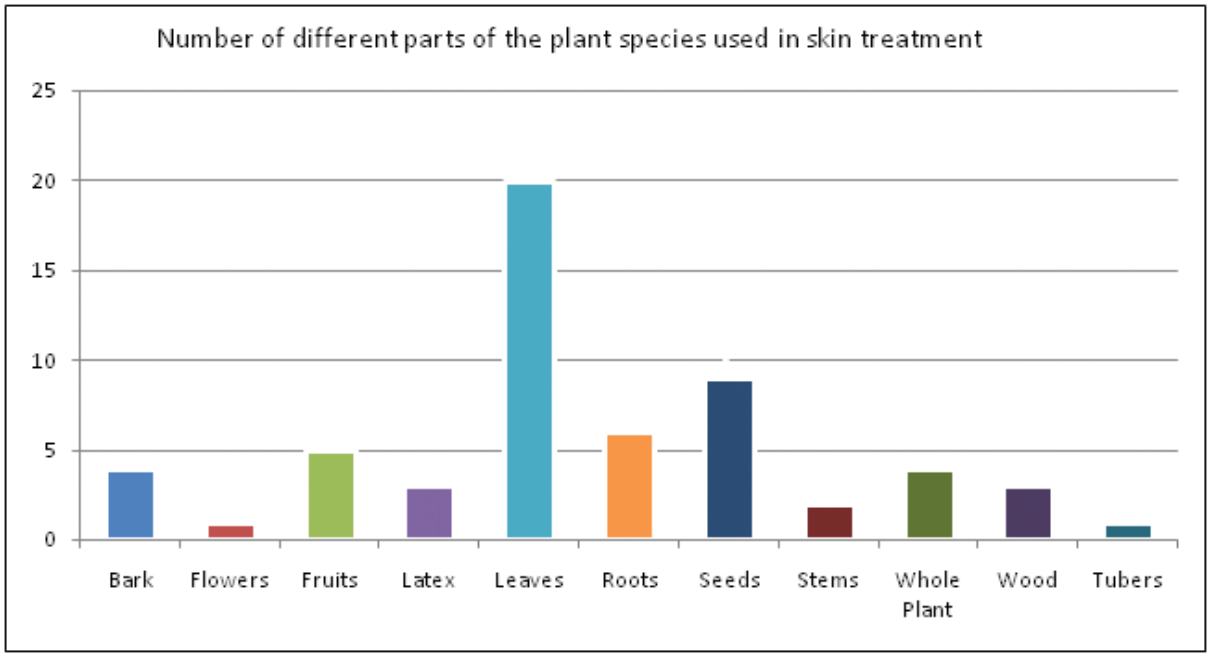

Fig. 1: Number of different parts of the plant species used

RESULTS AND DISCUSSIONS

Dumka district has the heritage of extensive phyto-therapy for the treatment of various diseases and promotion of health. Present study reveals a total of fifty- five significant ethno-medicinal plant species being utilized in skin treatment, by the indigenous and tribal herbal practitioners of the study area **(**Table 1). Number of species and percentage of the plant parts of different plant species used as ethno-medicine in treatment of skin problems has been presented in Table 2 and Figure 1. Plant species along with their local names, families, parts used and ethno-medicinal uses have been presented in the Table 3.

Table 1: Statistical synopsis of plant species utilized in skin treatment in Dumka

Groups	Families Number	Genera Number	Species Number
Pteridophyte	01	01	01
Dicotyledons	24	47	47
Monocotyledons	05	07	07
Total	30	55	55

Table 2: Statistical synopsis of the % of plant parts utilized in skin treatment:

Sl. No.	Plant Parts	(Actual Value)% Used
01	Bark	(04) 07%
02	Flowers	(01) 02%
03	Fruits	(05) 09%
04	Latex	(03) 05%
05	Leaves	(20) 34%
06	Roots	(06) 10%
07	Seeds	(09) 16%
08	Stems	(02) 03%
09	Tubers	(01) 02%
10	Whole Plant	(04) 07%
11	Wood	(03) 05%

Many of these plant species like, *Adhatoda vasica*, *Aegle marmelos*, *Aloe vera*, *Andrographis paniculata* (Patel *et al.* 2017), *Aristolochia indica*, *Bauhinia racemosa*, *Calotropis gigantea*, *Cyanodon dactylon* and *Momordica charantia* are also used to cure cough and cold, gastro-intestinal, fevers etc. in addition to skin problems. Some of these like *Aegle marmelos*, *Alocasia macrorrhiza*, *Azardirachta indica*, *Basella alba*, *Bauhinia racemosa*, *Citrus auruntifolia*, *Heliotropium*

A. Selling Ethno-medicines in *Hats*

B. *Argyreia nervosa*

C. *Coccinia grandis*

D. *Cocculu shirsutus*

E. *Calotropis gigantea*

F. *Heliotropium indicum*

G. *Holarrhena pubescens*

H. *Plumbago zeylanica*

I. *Tabernaemontana divaricata*

Table 3: Enumeration of ethno-medicines being used in skin treatment

Sl.No.	Scientific Name; [Family]; (Voucher- Specimen)	Local name	Parts used	Ethno-medicinal uses
1.	*Adenanthera pavonina* L.; [Fabaceae]; (AD - 186)	Ranjana, Badi Gumchi (IC), Rakt Chandan (H)	Seeds	The paste of the ground seeds is used twice a day to treat boils and inflammations.
2.	*Adhatoda vasica Nees.*; [Acanthaceae]; (AD-547)	Basok (S), Vasak (IC), Machraka (P)	Leaves	Poultice of the leaves is applied over fresh wounds, rheumatic joints and inflammatory swellings.
3.	*Adiantum capillus-veneris*; [Pteridaceae]; (AD-151).	Gheri-Bandha (S), Hansraj (IC)	Whole plant	Whole plant of about 100-150g is crushed & mixed with about 50ml mustard oil and then applied externally for 5-7 days to cure skin diseases in domestic animals.
4.	*Aegle marmelos* (L.) Correa; [Rutaceae]; (AD-548)	Bel (IC), Sinjo (S)	Leaves	Extract made from equal number of leaves of Bel &Sinduar (Vitex negundo L.) is applied topically over the acnes and pimples along with some Karpoor (Camphor).
5.	*Ageratum conyzoides* L.; [Asteraceae]; (AD-152)	Uchunti (IC), Vishamushti	Leaves	Juice of crushed leaves is applied on wounds & cuts of cattle to check bleeding. The aerial part of the plant is used mainly in leprosy and as bath in ecchymosis in humans too.
6.	*Alocasia macrorrhizos* (L.) G.Don.; [Araceae]; (AD- 101)	Kanda (S) (IC)	Stems	Extract of freshly cut stems is used on the skin to counter-affect itching caused after touching itchy or stinging plants such as sugar cane.
7.	*Aloe vera* (L.) Burm. *f.*; [Asphodelaceae];(AD - 243)	Gheekuari, Mosobari (S), Kattarvazha (P)	Leaves- pulp	Pulp of leaves is applied over tumours, cysts, inflamed parts and scalds. It is also effective in curing eczaema and burns.
8.	*Anacardium occidentale* L.; [Anacardiaceae]; (AD - 292)	Kaju (H) (IC)	Gums of barks	The gum of the bark is applied twice or thrice a day to cure leprosy, ringworm and ulcers.
9.	*Andrographis paniculata* (Burmf.) Nees.; [Acanthaceae]; (AD - 556)	Kalmeg (S), Kalmegh (IC) (H)	Leaves	Leaves ground with turmeric (Curcuma longa), is applied over cuts, wounds & skin diseases. Application should be continued till 7-10 days for cuts & wounds & 15-20 days for skin diseases.
10.	*Argemone mexicana* L.; [Papaveraceae]; (AD-136)	Siyalkanta (IC), Dhamoi, Pilli katail (H)	Roots & Stems	Crushed roots are applied over affected parts to treat eczema in domestic animals.
11.	*Argyreia nervosa* (Burm. f.) Bojer; [Convolvulaceae]; (AD- 134)	Kedok Arak (S), Bistarak, Vriddhadaruka (IC)	Leaves	Fresh leaves are tied over the boils for quick healing. Paste of the leaves is also applied over the wounds and is effective in rheumatism.

Contd.

Table 3 Contd.

Sl.No.	Scientific Name; [Family]; (Voucher- Specimen)	Local name	Parts used	Ethno-medicinal uses
12.	*Aristolochia indica* L.; [Aristolochiaceae]; (AD-309)	Godh (S), Iswarmul (H),	Roots	Pounded roots are rubbed with honey to cure leprosy.
13.	*Azadirachta indica* A. Juss.; [Meliaceae]; (AD- 112)	Neem (S) (H) (IC)	Whole plant	Paste of the leaves is applied on the affected part for getting relief from itching caused due to chicken-pox. It is also applied as face pack and other parts of the body to counter-effect the bacterial infections, pimples and rashes to condition the skin.
14.	*Azanza lampas* (Cav.) Alef.; [Malvaceae]; (AD - 339)	Bankapasi (H)	Roots & fruits	The roots and fruits are used in the treatment of wounds and sores.
15.	*Bambusa arundinacea* (Retz.) Willd.; [Poaceae]; (AD- 356)	Bans (H) (IC), Mah (S)	Leaves	Paste of the leaves is applied externally in the treatment of gout, wounds and for suppuration of boils.
16.	*Basella alba* L.; [Basellaceae]; (AD/SLB- 233)	Poi sag (IC), Porai (S), Pore (P)	Leaves	Pulp of leaves is applied over boils and ulcers to hasten suppuration. Extract of the leaves is mixed along with butter, soothes and cools burns and scalds.
17.	*Bauhinia racemosa* Lam.; [Fabaceae]; (AD-161)	Sin Arak (S), Koenar, Kachnar (H)	Barks	The decoction of barks is used to wash skin diseases and ulcers externally.
18.	*Bombax ceiba* L.; [Malvaceae]; (AD/SLB- 328)	Edel (S), Semal (H), Panjamaram (P)	Barks	To cure the patients suffering from small pox, 2 teaspoonfuls of paste of bark is administered orally, at an interval of 2-3 hours. It aids to outburst the small pox.
19.	*Buchanania lanzan* Spreng.; [Anacardiceae]; (AD/SLB- 569)	Tarop (S), Piyar (H)	Seeds oil	An ointment made of seeds relieves itching, prickly heat, rashes, skin spots, facial blemishes and other skin related problems.
20.	*Calotropis gigantea* (L.) Dryand.; [Apocynaceae]; (AD- 312)	Madar (H), Akaona (S), Erukku (P)	Leaves	Castor oil (Ricinus communis) is placed on the leaves & then warmed. It is applied in warm state over boil, blisters and skin diseases.
21.	*Citrus aurantiifolia* Swingle.; [Rutaceae]; (AD- 315)	Nimbu, Pati Lebu (IC)	Fruits	Dark marks of the skin on the elbow can be lightened by applying the juice of fruits twice a day regularly. Half a cup of juice of fruits along with 1 spoonful of glycerin in one cup cool, boiled milk, applied twice a day, effectively lessens the dark circles beneath eyes.

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Table 3 Contd.

Sl.No.	Scientific Name; [Family]; (Voucher- Specimen)	Local name	Parts used	Ethno-medicinal uses
22.	*Clitoria ternatea* L.; [Fabaceae]; (AD- 302)	Nil Baha (S), Aparajita (IC)	Leaves	Leaves are boiled in Til (Sesamum indicum L.) oil and applied, twice a day, to cure scabies in winter.
23.	*Coccinia grandis* (L.) Voigt; [Cucurbitaceae];(AD- 207)	Kundri (H), Tela Kucha (IC)	Leaves	Paste of the leaves is applied in the eruptions of skin.
24.	*Cocculus hirsutus* (L.) Diels; [Menispermaceae]; (AD- 326)	Patalgarudi (H), Jaljamini (IC)	Leaves	Paste of the leaves is applied to treat eczema.
25.	*Cordia dichotoma* G. Forst.; [Boraginaceae];(AD- 701)	Lasora, Bahubara(H), (IC), Buch (S)	Kernels	Paste of kernels is used in ringworm for about seven days thrice daily.
26.	*Curculigo orchioides* Gaertn.; [Hypoxidaceae]; (AD - 255)	Kali Musali (H), Turam (S)	Roots	Paste of roots is applied to hasten wound cure.
27.	*Curcuma longa* L.; [Zingiberaceae]; (AD- 113)	Haldi (H), Sasan (S)	Rhizomes	Paste of the rhizomes is applied all over the body either solitary or with the leaves of Neem, before bath, it makes the skin glow and also cures a number of skin diseases. Fresh juice along with lime or alum makes it a good dressing for sprains, bruises, wounds etc. (Das, 2014).
28.	*Cynodon dactylon* (L.) Pers.; [Poaceae]; (AD- 551)	Dub Ghas (H), Dubi Ghas (S)	Whole plants	Plant extract is boiled in four times that of the Ghee and is then applied over the pimples.
29.	*Eclipta prostrata* (L.) L.; [Asteraceae]; (AD- 217)	Bhangaraiya (H), Bhringraj (IC)	Roots	Paste of roots is applied externally as antiseptic to ulcers and wounds in cattle.
30.	*Eupatorium triplinerve* M.Vahl.; [Asteraceae]; (AD- 296)	Ayapan(H), (IC)	Leaves	Decoction of boiled leaves is used to wash chronic wounds and deep cuts twice as day, to heal it effectively.
31.	*Euphorbia thymifolia* L.; [Euphorbiaceae]; (AD- 679)	Chhoti- dudhi, Lal-Dudhiya (IC)	Latex	Latex of the plant is used in ringworm thrice daily.
32.	*Heliotropium indicum* L.; [Boraginaceae]; (AD- 212)	Hatisur (IC), Hati Sunda (S)	Leaves	Paste of equal part of leaves of the plant along with Wedelia chinensis, Cyanodon dactylon and Eclipta prostata is applied twice a day over the white scars of the burns to cure it.
33.	*Holarrhena pubescens* Wall. ex G. Don, [Apocynaceae]; (AD- 165)	Hat Baha (S), Kurchi, Kutaj (IC)	Bark and seeds	Decoction of the bark and seeds is taken internally in the early morning cures wound and boils.
34.	*Lantana camara* L.; [Verbeaceae];(AD- 624)	Putush (H)	Leaves	Leaves of the plant are used in leprosy, chicken pox, measles, etc.

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Table 3 Contd.

Sl.No.	Scientific Name; [Family]; (Voucher- Specimen)	Local name	Parts used	Ethno-medicinal uses
35.	*Millettia pinnata* (L.) Panigrahi; [Fabaceae]; (AD- 166)	Karanj (H), Kurunj (P)	Seeds	Seed oil is used to treat scabies and leukoderma.
36.	*Momordica charantia* L.; [Cucurbitaceae]; (AD- 107)	Karla, Kanchan arac (S), Karela (H)	Leaves	Two spoonful extract of leaves is taken along with luke warm water in morning in empty stomach to condition skin.
37.	*Ocimum tenuiflorum* L.; [Lamiaceae]; (AD- 111)	Tulsi (H) (IC), Tursi (S)	Whole plant	Leaves along with some salt and a few drops of lemon juice, is applied twice a day, over the affected parts by ringworms, cures it. Daily use of paste of leaves also conditions the skin.
38.	*Plumbago zeylanica* L.; [Plumbaginaceae];(AD- 318)	Chitrak (IC), Chitri(P)	Leaves & latex	Leaves of the plant are used externally in leprosy and other skin diseases of obstinate characters. Latex is also used in scabies.
39.	*Portulaca oleracea* L.; [Portulacaceae]; (AD- 232)	Kulfa	Leaves	The leaves & stems are applied topically in swellings, bruises, abscesses & boils.
40.	*Psoralea corylifolia* L.; [Fabaceae]; (AD- 705)	Babchi(IC), Bakuchi (H)	Seeds	Seeds of the plant are used in treating leukoderma.
41.	*Pueraria tuberose* (Willd.) DC.; [Fabaceae]; (AD- 226)	Tirra da (S) Patal Kohra (IC)	Tubers	Use of tubers regularly in diet, rejuvenates the skin and increases fairness and glow.
42.	*Ricinus communis* L.; [Euphorbiaceae]; (AD - 390)	Andi, Erand (H), Eradom (S), Elondi (P)	Roots	Paste of barks of roots along with some Curcuma longa rhizomes is applied over the itches and rashes twice a day to cure it.
43.	*Santalum album* L.; [Santalaceae]; (AD- 129)	Condon(S) Chandan (IC)	Wood	Paste of wood along with Haldi and Karpoor is applied twice or thrice a day, over the pimples and boils to cure it. It is also applied similarly on the face and body after bath and before going to bed to condition the skin and maintain its natural glow.
44.	*Semecarpus anacardium* L. f.; [Anacardiaceae]; (AD - 570)	Bhelwa (IC), Soso(S), Bale(P)	Fruits	Red- orange part of the fruits is considered good for various skin diseases.
45.	*Senegalia catechu* (L.f.) P.J.H. Hurter &Mabb.; [Fabaceae]; (AD - 234)	Khair (S) (IC)	Leaves & wood.	A tincture of the plant is used to treat bed sores and painful mammary glands.

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46.	*Senna alata* (L.) Roxb.; [Fabaceae]; (AD- 264)	Dadmari (IC)	Leaves wool"	Leaves are pounded, till "cottony is formed and then applied externally over to cure ringworm.
47.	*Shorea robusta* Roth; [Dipterocarpaceae]; (AD- 342)	Sal, Sakhua (IC), Sarjom (S), Karimaruthu (P)	Seeds	Oil of the seeds is applied all over the body to condition the skin. And to counter effect itches.
48.	*Spondias pinnata* (L.f.) Kurz.; [Anacardiaceae]; (AD- 371)	Ambra (S), Amra(IC), Ambaro(P)	Fruits	Ripened fruit's pulp is applied over wounds caused by prolonged water contacts, itches, cold cracks and eczemas.
49.	*Tabernaemontana divaricata* R.Br. ex Roem. &Schult.; [Apocyanaceae]; (AD - 258)	Tagar (IC), Sada Baha (S)	Wood	It is also applicable similar to Santalum album to cure pimples and acnes.
50.	*Tectona grandis* L.f.; [Verbenaceae]; (AD - 301)	Sagwan(H), (IC)	Seeds	Oil of the seeds is applied twice or thrice a day, externally to cure itches.
51.	*Terminalia elliptica* Willd.; [Combretaceae]; (AD -564)	Ason (IC), Atnak(S)	Barks	Ash of about 2 inches brunt bark mixed with til (*Sesamum indicum* L.) oil is applied topically to cure itches.
52.	*Trichosanthes dioica* Roxb.; [Cucurbitaceae]; (AD - 380)	Patal, Parwal (H), (IC)	Fruits	Painful swellings during cutting of nails with pus can be cured by placing the finger into the grilled (half brunt) fruits, twice or thrice a day.
53.	*Triticum aestivum* L.; [Poaceae]; (AD - 253)	Genhu (H), Gom(IC)	Seed grains (Cryopsis)	Boiled seeds husk and flour of seeds are applied to clean and condition the dry skin.
54.	*Vachellia nilotica* (L.) P.J.H. Hurter &Mabb.; [Fabaceae]; (AD - 288)	Babla (S) (IC), Babul (H)	Barks	8-10 g of pounded barks are boiled in water and latter is then use to wash injured mammary glands caused during feeding babies among nursing mothers.
55.	*Woodfordia fruticosa* (L.) Kurz; [Lythraceae]; (AD - 335)	Dhai (IC), Icak (S)	Flowers	Paste of *Dhai* flowers and Lodh (Symplocos racemosa (Roxb.)) bark in equal amount, is applied topically over pimples and acnes to cure it.

*(S)- Santal Community, **(P)- Paharia Community, ***(IC)- Indigenous Communities like Mahalis, Bhumijs, ****(H) Hindietc.

indicum, Momordica charantia, Pueraria tuberosa, Spondia spinnata and *Triticum aestivum* are sold in the local markets called as *Haats* or *Hatias* and are used either as vegetables or leafy vegetables which are also main source of earning their livelihood (Das, 2018). Others like *Aegle marmelos, Azardirachta indica, Calotropis gigantea, Citrus ×auruntiifolia, Clitoria ternatea, Curcuma longa, Cyanodon dactylon* and *Ocimum sanctum* do possess religious significance too (Das and Bondya, 2015).

Ethno-medicines or the folk medical claims are considered an important component of traditional knowledge which is being practiced by a handful of herbal healers, older family members and knowledgeable women of the communities. Ethno-

medicines are used either singly or in association with two or more species to prepare the drugs to cure specific diseases of skin and promoting health (Jain and Tarafder, 1970).

CONCLUSION

Ethno-medicinal plants utilized by these herbal healers can no doubt discover new drugs or escort molecules for the development of new drugs, medicines, provided the formulations and data should have integrated scientific approaches (Mukherjee *et al.*, 2015). At the same time, efforts should be made to recognize Intellectual Property Rights of these ethnic herbal medicinal practitioners, to sustainably utilize their precious knowledge and conserve these for our future generation (Borathakur and Gogoi, 1994). Integrated scientific approaches can meet the emergent future requirement of different significant drugs and medicines.

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REFERENCES :

Anonymous, 1948-1976. *The Wealth of India. Raw Materials*. C.S.I.R., New Delhi.

Borathakur, S.K. and P. Gogoi. 1994. Conservation of Biological Diversity-A cultural tradition of the tais. *The Tais*.**1**:189-199.

Chopra, R.N., S.L.Nayar and I.C. Chopra. 1956. *Glossary of Indian Medicinal Plants*. C.S.I.R., New Delhi.

Das, A. 2014. Some important medicinal herbs used in our kitchen. *Resonance - National Journal of Value Education,* **2**(2): 41-48.

Das, A. and S.L. Bondya. 2015. Tribal Communities as the conservator of traditional medicinal knowledge in Dumka district, Jharkhand, India. In *Biodiversity, Conservation and Sustainable Development,* **1**:70-76. Issues and Approaches (ed.: P. Jha), New Academic Publishers, New Delhi.

Das, A. 2018. Ethno-botanical uses of wild fruits of Santal Paraganas (Jharkhand). *International Journal of Minor Fruits, Medicinal and Aromatic Plants,* **4**(2): 31-38.

Haines H.H. 1921-1925. *The Botany of Bihar and Orissa* I-IV (BSI, Calcutta).

Jain, S.K. 1968. *Medicinal Plants*. National Book Trust, New Delhi.

Jain, S.K. and R.R. Rao. 1977. *A Handbook of field and Herbarium methods*. Today & Tomorrow's Printers and Publishers, New Delhi.

Kirtikar, K.R.and B.D.Basu. 1935. *Indian Medicinal Plants* Vols. 1-4 (2nd ed.) Bishen Singh Mahendra Pal Singh, Dehra Dun and Periodical Experts, Delhi.

Maheshwari, P. and U. Singh. 1965. *Dictionary of Economic Plants in India*. I.C.A.R., New Delhi.

Mukherjee, P., S.L.Bondya and A. Das. 2015. Traditional Knowledge: Best medicines, a way forward. In *Recent Advances in Ethnobotany,* **P.**191-196. (eds.: Sanjeev Kumar), Deep Publications, New Delhi,

Patel,V.D. M.M. Patel, H.V. Pandya and S.N. Saravaiya 2017. Effect of integrated nutrient management on yield and economics of Kalmegh (*Andrographis paniculata* Wall ex Nees.). *International Journal of Minor Fruits, Medicinal and Aromatic Plants,* **3**(1): 1-4.

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Anonymous. 1979. Mango varieties of West Bengal. Technical Bulletin No. 1.

Department of Horticulture, Faculty of agriculture, Bidhan Chandra Krishi Viswavidyalaya. Pp.52.

Chapter in book : Singh, Harminder, Thakur Anirudh and Jawandha, S. K. 2010a. Varietal improvement and production technologies in peach. In. Temperate fruits in subtropics. WS Dhillon (ed). Department of Horticulture, Punjab Agricultural University, Ludhiana pp 5-8.

Proceedings: Blake, M.A. 1932. The J.H. Hale as a parent in peach crosses. Proc. Am. Soc. Hort. Sci., 29:131-136.

Monet, R. 1979. Transmission génétique du caractère 'fruit doux' chez le pêcher. Incidence sur la selection pour la qualité. In: Proceedings of Eucarpia Fruit Section Symposium. Tree Fruit Breeding. INRA, Angers, France, pp. 273-276.

Bulletin: Gray, P. 1914. The compatibility of insecticides and fungicides. Monthly bulletin of California, July, 1914.

Annual meetings: Schenck, N.C. 1965. Compatibility of fungicides with insecticides and foliar nutrients. 57th annual Meeting of American Phytopathological Society, 3-7 October, 1965.

Reports: Anonymous, 1971. Investigations of insects pests of sorghum and millets. Final Technical report, 1965-70, IARI, New Delhi, pp.157.

Annual report: Anonymous, 2010. Annual Report for 2010-11, Faculty of Horticulture, Bidhan Chandra Krishi Viswavidyalaya, Monhanpur, Nadia, West Bengal, India. Pp.80-85.

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