Pak. J. Bot., 55(2): DOI: http://dx.doi.org/10.30848/PJB2023-2(4)

QUALITATIVE PHYTOCHEMICAL SCREENING OF SOME MEDICINALLY IMPORTANT ALPINE PLANTS FROM MASTUJ VALLEY, HINDU KUSH RANGE PAKISTAN

FARRUKH HUSSAIN[1*], SYED MUKARAM SHAH[2], KHALID AZIZ[1], SAPNA RAISHAM[1] AND SHABIR AHMAD[1]

[1]*Institute of Biological Sciences, Sarhad University of Science & Information Technology, Peshawar, Pakistan*
[2]*Centre of Plant Biodiversity, University of Peshawar, Pakistan*
[*]*Corresponding e-mail: farrukh.biotech@suit.edu.pk*

Abstract

Qualitative phytochemical screening of 24 medicinally important alpine plants from Mastuj Valley, Hindu Kush Range, Pakistan was carried out for the detection of alkaloids, saponins, tannins, and oil contents at three phenological stages: Pre-reproductive (young vegetative stage), Reproductive (flowering/fruiting stage) and Post-reproductive stages (fruit/seed maturation and dispersal stage). The selected plants were distributed among, 5 tree, 8 shrub, 7 herbaceous and 4 grassy species belonging to 16 families. Phytochemical screening indicated that 15 species contained all the 4 chemicals, 5 species had 3, 3 species had 2 and one species had only oil. Alkaloids and saponins were present in 21 species. Tannins and oils were detected in 20 species. Alkaloids were recorded in 12, 8 and 20 species in pre-reproductive, reproductive and post reproductive stages, respectively. Saponins were recorded in 21, 19 and 16 species respectively at pre-reproductive, reproductive and post reproductive stages. Pre-reproductive, reproductive and post reproductive stages respectively had tannins in 18, 15 and 16 species. Oils were recorded in 14, 15 and 18 species in the three successive phenological stages. This preliminary study suggests further quantitative analysis for the screened and other phytochemicals in different plants, their various parts to exactly pin-point the quantity of these useful chemicals under varied environmental conditions by using different solvents.

Key words: Phytochemicals, Phenology, Alpine plants, Mastuj-Hindu Kush Range.

Introduction

Phytochemicals are naturally occurring biologically active chemical compounds found in various parts of plants. These phytochemicals are distinguished into primary and secondary metabolites. Primary metabolites including chlorophyll, proteins sugar and amino acids are directly involved in the plant growth and metabolic functions. Secondary metabolites are derived from the primary metabolic pathways. These metabolites have antifungal, antimicrobial, anti-inflammatory, anticancer, anti-tumor, anti-hypertensive and anti-proliferative nature. Secondary metabolites are also used as agrochemicals, pharmaceuticals, flavor, fragrance, food additives and pesticides (Fazili *et al*., 2022; Twaiji *et al*., 2022).These chemicals also protect plants from diseases, pollution hazards, grazing and other environmental damages. Their nature and concentration vary among plants and plant parts under a set of climatic and phenological conditions. Plants are used for medicinal purposes since time immemorial. Some 80% of the populations in developing world still rely on the traditional herbal healthcare system (Balamurugan *et al*., 2019). Khan *et al*., (2021) hinted upon the possibility of potent role of secondary chemicals in fighting COVID-19. Afzal *et al.,* (2022) reported that total phenolics, flavonoids, tannins, alkaloids, saponins and terpenoids contents varied in different plant extracts of *Pseudocaryopteris foetida*. Lydia *et al*., (2022) after phytochemical screening of 53 Philippine plants stated that tannins were present in 50 plants, alkaloids in 43 plants, glycosides in 33 plants, flavonoids in 31 species and saponins in 8 species. Otari *et al*., (2022) investigated phenolics, glycosides, terpenes, steroids and fatty acids in *Barleria terminalis* and *Calacanthus grandiflorus*. The extracts from leaves of *Neoglaziovia variegatea* (Gomes *et al*., 2022) collected monthly round the year contained varying concentrations of anthocyanins, anthraquinones, anthracene derivatives, flavonoids, tannins, terpenes and steroids. Sharifi-Rad *et al*., (2022) identified 49 compounds in *Teucrium polium* having differences in the phytochemicals based on the growth and the phenological stages of plants. Maximum phenolics and sapnins contents were recorded at flowering stage. Aljaiyash *et al*., (2022) while evaluating the yield, chemical composition and biological activities of essential oil from *Thymus maroccanus* at different phenological stages observed that carvacrol and p-cymene gradually increased from the lowest amount at the pre-flowering stage to the highest level at post-flowering stage. Pereira *et al*., (2022) reported that the alkaloids, organic acids, flavonoids, phenols and tannins in *Montrichardia linifera* leaf extracts varied according to the place of collection and solvent used. Rubio *et al*., (2022) reported similar phytochemical compositions from naturally growing plants and micro propagated explants. Akinmoladun *et al*., (2022) identified various phytochemicals from *Chrysophyllum albidum*. Phytochemical screening of methanolic extract from leaves of *Chapta lianutans* revealed the presence of alkaloids, coumarins, tannins, phenolics, flavonoids, and free steroids (deSouza *et al*., 2022). Abbas *et al*., (2021) reported biologically active phenolics and flavonoids from *Seriphidium oliverianum*. Abeysinghe *et al*., (2021) identified alkaloids, flavonoids, saponins, tannins, terpenes and steroids with antioxidant and antimicrobial activities from *Murraya koenigii* and *Micromelum minutum*. Alkaloids, saponins, flavonoids, steroids, tannins, phenolics, glycosides, oil and fats were detected in *Plumbago indica* (Bashir & Kumar, 2021), *Ocimum sanctum* (Gwari *et al*., 2021) and *Euphorbia helioscopia* and *Rumex dentatus* with antimicrobial, analgesic and antioxidant properties (Mehmood *et al*., 2021). Alkaloids were also reported in *Acorus calamus, Senna alata, Solanum torvum* and *S. trilobatum* (Mohan *et al*., 2021). Mokua *et al*., (2021) identified alkaloids, saponins, tannins, phenols and flavonoids from 14 plants locally used for treating snakebite in Kenya. Yu *et al*., (2021) reported several phytochemicals in fruit plants with varying concentrations among the tested species. Phytochemical screening of *Echinops amplexicaulis, Ruta chalepensis* and *Salix subserrata* (Marami *et al*., 2021); and ***P****rosopis africana, Curcuma longa* and *Psorospermum febrifugum* (Elaigwu *et al*., 2020) revealed alkaloids, saponins, phenols, tannins, flavonoids, phytosterols, steroids, terpenoids, and cardiac glycosides. Iheagwam *et al*., (2019) observed that natural ripened mangoes had higher carotenoids, phenols and terpenes compared to alkaloids, tannins and flavonoids. Many recent contemporary studies on phytochemical screening revealed the presence of various secondary plant metabolites varying in occurrence and concentration based on the plant part, species, climate, growth, phenological stage and type of solvent used for extraction (Afzal *et al*., 2022; Twaij *et al*., 2022; Sreenivasulu *et al*., 2022; Wawrosch & Zotchev, 2021; Onefeli*et al*., 2021; Feudjio *et al*., 2020; Choukri *et al*., 2020; Reaisi *et a1*., 2019).

The survey of recent literature strongly favours the importance of phytochemical screening of medicinal plants as a primary source for commercial exploitation. It is also obvious that no such work is available on the alpine plants of Mastuj, Hindu Kush Range, Pakistan. The present endeavour was, therefore, aimed to chemically evaluate 24 common alpine plants being locally used in the traditional health care system (Hussain *et al*., 2007; Shah & Hussain, 2012) in the Mastuj Valley, Pakistan. These preliminary findings will pave future way for phytochemists, biotechnologists, herbalists and pharmaceutical industries for exploring new drugs for combating various diseases.

Materials and Methods

Healthy shoots/leaves of 24 alpine plant species were collected at three phenological stages, *i.e.* pre-reproductive stage (young vegetative stage), reproductive stage (flowering/fruiting stage) and post-reproductive stage (fruit/seed maturation and dispersal stage) during summer from Mastuj Valley. The plant material was shade dried for 21 days and powdered. These were qualitatively screened for the presence of alkaloids, saponins, tannins and oils following the standard methods (Banu & Cathrine, 2015; Balamurugan *et al*., 2019; Trease & Evans, 2009). There were, 5 tree, 8 shrub, 7 herbaceous and 4 grassy species of 16 families. For alkaloids, 2 drops of Mayer's reagent was added to extract; a creamy or white precipitate indicated the presence of alkaloids. It was confirmed by adding 2ml HCl to 5 ml extract, followed by 1 ml of Dragendroff's reagent. The appearance of orange or red precipitate indicated the presence of alkaloids. Saponins were detected by vigorous shaking of 5 ml of extract with few ml distilled water. The formation of froth indicated saponins. The resultant froth with few drops of olive oil was vigorously shaken that formed emulsion, which further confirmed the presence of saponins. Tannins were deducted by adding few drops of neutral ferric chloride solution to 5 ml of extract. The appearance of dark green colour showed the presence of tannins. The presence of oils was checked by spot test. Small amount of extract was pressed between 2 filter papers. The appearance of spot showed the presence of oils. Further testing was done by saponification. Few drops of 0.5N alcoholic KOH and few drops of phenolphthalein were added and heated for 2 hours. The formation of soap or partial neutralization of alkali indicated the presence of fixed oils or fats.

Results and Discussion

The qualitative screening of four secondary metabolites in 24 medicinally important alpine plants of Mastuj Valley indicated that alkaloids and saponins were present in 21 species. Tannins and oils were detected in 20 species. Fifteen species contained all the 4 phytochemicals, 5 species had 3 phytochemicals and 3 species had 2 phytochemicals. Ibrahim *et al*., (2021) observed that all the tested plants had varied amounts of alkaloids, saponins and tannins. In the present case 24 species showed variations in types of phytochemicals among different species and at various phenological stages. The results agree with Lydia *et al*., (2022), who reported that tannins were present in 50 plant species, alkaloids in 43 species, glycosides in 33 species and saponins in 8 species. Based on the phenological stages alkaloids were respectively recorded in 12, 8 and 20 species at pre-reproductive, reproductive and post reproductive stages. Saponins were accordingly present in 21, 19 and 16 species in pre-reproductive, reproductive and post reproductive stages. The pre-reproductive, reproductive and post-reproductive stages sequentially had tannins in 18, 15 and 16 species. Oils were recorded in 14, 15 and 18 species in the three successive phenological stages. Based on the age and plant part, leaves of *Cola millenii* had none of the tested secondary compounds including tannins, saponins, steroids and alkaloids etc (Onefeli *et al*., 2021), but its fruit had the highest flavonoid contents. Verdecia *et al*., (2021) also observed that primary compounds decreased with the advancing age; while secondary compounds enhanced with maturity. This also supported the present findings as the presence/absence of various phytochemicals depended on phenological stage. Feudjio *et al*. (2020) also reported that phenology affected quantitative and qualitative values of phytochemicals in plants. They further stated a decrease of phytochemicals during flowering and fruiting stage. Adegbaju *et al*., (2020) is also of the view that developmental stages influence the physiology and chemistry of plants. Phenological stage is the growth stage that varies with season. The Pre-reproductive stage is the young seedling stage in annuals and pre-flowering stage in perennial plants. Reproductive stage is the flowering and fruiting stage in annuals and perennial species; while Post-reproductive phase represents post-flowering, fruiting and dispersal of seeds and fruits period. It is the completion of life cycle in annual plants; while in perennial species it represents dormant stage. These stages are correlated to the seasonal changes in each locality under a set of climatic conditions. Keeping this view in mind, individual phytochemicals are discussed below.

1. Alkaloids: Alkaloids are important in traditional human healthcare system due to their antibacterial, antifungal, anti-parasite, antimalarial, analgesic, anti-fibrillation and anti-tumor activities (Fazili *et al*., 2022; Twaij *et al*., 2022). Alkaloids were present in 21 species, which were distributed respectively in 12, 8 and 20 species at pre-reproductive, reproductive and post reproductive stages. The present study highlights the presence of alkaloids in *Betula utilis* at all the three phonological stages (Table 1). It was present at reproductive stage in *Tamarix sp.* and at post-reproductive stage in *Hippophae rhamnoides.* It was not detected at pre-reproductive stage in *Elaeagnus angustifolia* and *Juniperus excelsa.* Among the shrubs (Table 2), alkaloids were present at all the phenological stages in *Acantholimon longiscapum, Ephedra gerardiana, Sophora mollis* and *Berberis lyceum.* However, it was completely absent in *Capparis spinosa*. It remained below the deductible level in *Myrtama elegans* and *Rosa webbiana* at pre-reproductive stages. *Datura stramonium, Glycyrrhiza glabra, Mentha longifolia, Rheum emodi* and *Salvia nubicola* among the herbs possessed alkaloids at all the three phenological stages (Table 3). Alkaloids were also noticed in the post-reproductive stages of *Nepeta cataria* and *Verbascum thapsus*. Among the grasses (Table 4), alkaloids were present in *Cynodon dactylon* and *Phragmites karka* at all the three phenological stages; but it tested negative in *Calamogrostris pseudophragmites* and *Saccharum spontaneum* at the three phenological stages. Alkaloids were detected in 21 species. Of them, it was recorded 12, 18 and 20 times respectively at pre-reproductive, reproductive and post-reproductive stages. Our results agreed with many workers who reported the presence of alkaloids along with other phytochemicals that differed from species to species and collection site (Pereira *et al*., 2022), and season (deSouza *et al*., 2022; Gomes *et al*., 2022; Fazili *et al*., 2022). The present findings also agree with those of Sharifi-Rad *et al.,* (2022), who stated that alkaloid contents of *Teucrium polium* differed in shoots and roots at various phenological stages. The maximum alkaloids contents were achieved at flowering stage. Mohan *et al.,* (2021) also spotted alkaloids in all the tested medicinal plants, which agreed with the present findings. Similarly, Mehmood *et al.,* (2021) also observed the presence of alkaloids in *Euphorbia helioscopia* and *Rumex dentatus*. Marami *et al.,* (2021) concluded that all the analyzed phytochemicals were present in all the plants tested by them. This also strengthened the present findings as we recorded the presence of four tested phytochemicals in 21 investigated species. Many available studies indicate the presence of alkaloids in various plants or their parts (Gwari *et al*., 2021; Bashir & Kumar, 2021; Shukla *et al*., 2021) that are in line with the present study.

Table 1. Some phytochemicals in alpine medicinal tree species of Mastuj Valley, Hindu Kush Range, Pakistan.

Plant species	Phenological Stage		Alkaloids	Saponins	Tannins	Oils
1. ***Betula utilis* D. Don**						
	i.	Pre-reproductive stage	+	+	-	-
	ii.	Reproductive stage	+	+	+	+
	iii.	Post-reproductive stage	+	+	+	+
2. ***Elaeagnus angustifolia* L.**						
	i.	Pre-reproductive stage	-	+	+	-
	ii.	Reproductive stage	+	+	+	+
	iii.	Post-reproductive stage	+	+	+	+
3. ***Hippophae rhamnoides* L.**						
	i.	Pre-reproductive stage	-	+	+	+
	ii.	Reproductive stage	-	+	+	+
	iii.	Post-reproductive stage	+	-	+	+
4. ***Juniperus excelsa* M. Bieb.**						
	i.	Pre-reproductive stage	-	+	+	+
	ii.	Reproductive stage	+	+	+	+
	iii.	Post-reproductive stage	+	+	+	+
5. ***Tamarix* sp.**						
	iv.	Pre-reproductive stage	-	+	+	+
	v.	Reproductive stage	+	+	-	-
	vi.	Post-reproductive stage	-	+	-	+

Table 2. Some phytochemicals in alpine medicinal shrubby species of Mastuj Valley, Hindu Kush Range, Pakistan.

Plant species		Phenological Stage	Alkaloids	Saponins	Tannins	Oils
1. ***Acantholimon longiscapum*** **Bokhari**						
	i.	Pre-reproductive stage	+	+	-	-
	ii.	Reproductive stage	+	+	-	-
	iii.	Post-reproductive stage	+	+	+	+
2. ***Berberis lyceum*** **Royle**						
	i.	Pre-reproductive stage	+	-	+	+
	ii.	Reproductive stage	+	-	+	+
	iii.	Post-reproductive stage	+	-	+	+
3. ***Capparis spinosa*** **L**						
	i.	Pre-reproductive stage	-	+	+	-
	ii.	Reproductive stage	-	+	+	-
	iii.	Post-reproductive stage	-	+	+	-
4. ***Ephedra gerardiana*** **L.**						
	i.	Pre-reproductive stage	+	+	-	+
	ii.	Reproductive stage	+	+	+	+
	iii.	Post-reproductive stage	+	+	+	+
5. ***Myrtama elegans*** **(Royle) Ovcz. & Kinzik**						
	i.	Pre-reproductive stage	-	+	+	+
	ii.	Reproductive stage	+	+	+	-
	iii.	Post-reproductive stage	+	-	+	+
6. ***Rosa webbiana*** **Wall ex Royle**						
	i.	Pre-reproductive stage	-	+	+	-
	ii.	Reproductive stage	+	+	+	-
	iii.	Post-reproductive stage	+	+	+	+
7. ***Sophora mollis*** **(Royle) Baker**						
	i.	Pre-reproductive stage	+	+	+	+
	ii.	Reproductive stage	+	+	+	+
	iii.	Post-reproductive stage	+	+	+	+
8. ***Artemisia parviflora*** **Roxb.**						
	i.	Pre-reproductive stage	-	+	+	-
	ii.	Reproductive stage	+	-	-	-
	iii.	Post-reproductive stage	+	-	-	-

2. Saponins: Saponins are therapeutically important due to their hypolipidemic and anticancer activities. Cardiac glycosides and steroidal saponins are used commercially for the production of sex hormones. Saponins, present in 21 species, were accordingly recorded in 21, 19 and 16 species in pre-reproductive, reproductive and post reproductive stages. Saponins were present at all the phenological stages in the tree species (Table 1). Saponins were also detected in *Hippophae rhamnoides* at pre- and reproductive stages. Among the shrubs (Table 2), saponins were present at all the three phenological stages in *Acantholimon longiscapum*, *Capparis spinosa, Ephedra gerardiana, Rosa webbiana* and *Sophora mollis*. *Berberis lyceum* tested negative for saponins at all the three phenological stages. *Myrtama elegans* was positive for saponins at pre-reproductive and productive stages. Saponins were recorded in *Artemisia parviflora* at pre-reproductive stage. Among the herbs (Table 3), saponin was present in the three phenological stages in *Datura stramonium*, *Glycrhiza glabra*, *Mentha longifolia*, *Rheum emodi* and *Verbascum thapsus*. *Salvia nubicola* had saponins at pre- and reproductive stages. Saponins were noticed in *Nepeta cateria* only at pre-reproductive stages. Saponins were absent in *Calamogrostris pseudophragmites* and *Phragmiteskarka*at all the phenological stages; but on the contrary, *Cynodon dactylon* and *Saccharum spontaneum* were positive for saponins at all the three growth stages (Table 4). Akinmoladun *et al.,* (2022) reported the absence of saponins in *Chrysophyllum albidum*, which supports the present findings. Sharifi-Rad *et al.*, *(*2022) recorded the highest and lowest saponin contents in the aerial parts of *Teucrium polium* respectively at flowering and at immature stages. Similar to the present findings, Mehmood *et al.,* (2021) also observed tannins in *Euphorbia helioscopia*. Marami *et al.,* (2021) concluded that saponins were present in all the plants analyzed by them. In our case, 87.5% plants contained saponins. Many recent studies support the presence of saponins in various plants (Gwari *et al*., 2021; Bashir & Kumar, 2021; Mansoori *et al*., 2020; Elaigwu *et al*., 2020; Sulaiman *et al*., 2020) that are in line with the present findings.

Table 3. Some phytochemicals in alpine medicinal herbaceous species of Mastuj Valley, Hindu Kush Range, Pakistan.

Plant species	Phenological Stage		Alkaloids	Saponins	Tannins	Oils
1. ***Datura stramonium*** **L.**						
	i.	Pre-reproductive stage	+	+	+	+
	ii.	Reproductive stage	+	+	+	+
	iii.	Post-reproductive stage	+	+	+	+
2. ***Glycyrrhiza glabra*** L.						
	i.	Pre-reproductive stage	+	+	+	-
	ii.	Reproductive stage	+	+	+	-
	iii.	Post-reproductive stage	+	+	+	-
3. ***Mentha longifolia*** **(L.) Huds.**						
	i.	Pre-reproductive stage	+	+	+	-
	ii.	Reproductive stage	+	+	+	+
	iii.	Post-reproductive stage.	+	+	+	+
4. ***Nepeta cataria*** L.						
	i.	Pre-reproductive stage	-	+	+	+
	ii.	Reproductive stage	-	-	+	+
	iii.	Post-reproductive stage	+	-	+	+
5. ***Rheum emodi*** **Wall ex Meissn.**						
	i.	Pre-reproductive stage	+	+	+	-
	ii.	Reproductive stage	+	+	-	-
	iii.	Post-reproductive stage	+	+	-	-
6. ***Salvia nubicola*** **Wall ex Sweet**						
	i.	Pre-reproductive stage	+	+	+	-
	ii.	Reproductive stage	+	+	+	+
	iii.	Post-reproductive stage	+	-	+	+
7. ***Verbascum thapsus*** L.						
	i.	Pre-reproductive stage	-	+	+	+
	ii.	Reproductive stage	-	+	-	-
	iii.	Post-reproductive stage	+	+	-	-

Table 4. Some phytochemicals in alpine grasses of Mastuj Valley, Hindu Kush Range, Pakistan.

Plant species	Phenological Stage		Alkaloids	Saponins	Tannins	Oils
1. ***Calamogrostris pseudophragmites*** **Hall. f. Koeler**						
	i.	Pre-reproductive stage	-	-	-	+
	ii.	Reproductive stage	-	-	-	+
	iii.	Post-reproductive stage	-	-	-	+
2. ***Cynodon dactylon*** **(L.) Pers.**						
	i.	Pre-reproductive stage	+	+	-	+
	ii.	Reproductive stage	+	+	-	+
	iii.	Post-reproductive stage	+	+	-	+
3. ***Phragmites karka*** **(Retz.) Trin ex Steud.**						
	i.	Pre-reproductive stage	+	-	-	+
	ii.	Reproductive stage	+	-	-	+
	iii.	Post-reproductive stage	+	-	-	+
4. ***Saccharum spontaneum*** **L.**						
	i.	Pre-reproductive stage	-	+	-	+
	ii.	Reproductive stage	-	+	-	+
	iii.	Post-reproductive stage	-	+	-	+

3. Tannins: Tannins are water and alcohol soluble phenolic compounds of high molecular weight, with common distribution in various parts of plants. Tannins were detected in 20 species. The pre-reproductive, reproductive and post reproductive stages sequentially had tannins in 18, 15 and 16 species. The present study reported tannins at three phenological stages of tree species (Table 1) including *Betula utilis*, *Elaeagnus angustifolia, Hipophae rhamnoides* and *Juniperus excelsa* Tannins were absent in *Tamarix sp.* at reproductive and post-reproductive stages. Amongst shrubs (Table 2), *Berberis lyceum*, *Capparis spinosa*, *Myrtama elegans*, *Rosa webbiana* and *Sophora mollis* contained tannins at all the three phenological stages. It was present at post-reproductive stage in *Acantholimon longiscapum;* and at reproductive and post-reproductive stages in *Ephedera gerardiana*. *Artemisia parviflora* contained tannins at pre-reproductive phase. Among the herbs (Table 3), tannins were recorded in *Datura stramonium, Glycyrrhiza glabra, Mentha longifolia, Nepeta cataria* and *Salvia nubicola* at all the phenological stages. Whereas, *Rheum emodi* and *Verbascum thapsus* had tannins at pre-reproductive stages only. Tannins were completely absent in all the 4 tested grasses at all the growth stages (Table 4). Like the present study, tannins were present in *Montrichardia* (Pereira *et al*., 2022) and *Chrysophyllum albidum* (Akinmoladum *et al*., 2022) that varied in concentration due to locality. The findings of Aljaiyash *et al.,* (2022) are in line with the present findings as they also stated that phytochemicals present in *Thymus marocannus* increased from pre-flowering to post-flowering stages. Similarly, Yu *et al.,* (2021) also achieved highest tannin contents in pomegranate among various food and fruit plants. They recorded that tannins varied from absent to various amounts among the tested alpine plants at various phenological stages. This also strengthened our findings regarding the role of phenology in the absence/presence of a particular secondary chemical. Various studies also reported tannins in various plants (Gwari *et al*., 2021; Nagano & Batalini, 2021; Mehmood *et al*., 2021; Bashir & Kumar, 2021; Mansoori *et al*., 2020; Farag *et al*., 2020; Elaigwa *et al*., 2020), which support the present findings.

4. Oils: Oils were detected in 20 species, which successively appeared in 14, 15 and 18 species in pre-reproductive, reproductive and post-reproductive phenological stages. Amongst the tree species (Table 1), oil was detected in *Hipophae rhamnoides* and *Juniperus excelsa* at all the three phenological stages. It was also present at reproductive and post-reproductive stages in *Betula utilis* and *Elaeagnus angustifolia,* While*, Tamarix sp.* contained oils in pre- and post-reproductive stages. Within the shrubs (Table 2), oil was present at all the three phenological stages in *Berberis lyceum*, *Ephedera gerardiana* and *Sophora mollis;* but oil was absent at all the three growth stages in *Artemisia*. Oil was detected at post-reproductive stage in *Acantholimon longiscapum* and *Rosa webbiana* and at pre- and post-reproductive stages in *Myrtama elegans.* Among the herbs (Table 3), oils were spotted at all the three phenological stages of *Datura stramonium,* and *Nepeta cataria*. On the other hand, *Glycyrrhiza glabra* and *Rheum emodi* tested negative at all the three phenological stages for oil contents. *Mentha longifolia* and *Salvia nubicola* had oils at reproductive and post-reproductive stages. *Verbascum thapsus* had oil only at the pre-reproductive stage. All the three phenological stages of the four analyzed grasses (Table 4) contained oils. Aljaiyash *et al.,* (2022) observed that oil contents increased from pre-flowering to flowering stage; but declined in the post-flowering stage. Mollaei *et al.,* (2020) demonstrated that the maximum yield of *Zosima* oil was achieved at the late-mature/ripe seeds. The findings also agree with those of Reaisi *et al*., (2019), who achieved highest yield of oil at seed filling stage and demonstrated a correlation between altitude, phenology and oil contents and their composition.

Conclusions

Plants exhibit phytochemical diversity due to climatic, edaphic and phenologic variation. Same was true in the present study. Phytochemical screening is desirable for the discovery of therapeutic agents and new sources of medicinal material. Various secondary compounds including tannins, oils, gums, flavonoids, saponins and essential oils are medicinally important that act as precursors for the synthesis of complex chemical substances. The chemical composition of plants varies according with growth stage due to physiological demands. The pre-reproductive phase is vegetative seedling stage, which is sequentially followed by reproductive (flowering & fruiting) and post-reproductive (maturation of fruits and their dispersal) stages. The annual plants complete their life cycle after shedding their seeds and fruits; while for perennial species this stage means advancing towards dormant stage. The results may help in finding the suitable stage for harvesting of medicinal plant for a particular chemical. The findings can also be helpful in regulating the time of grazing as Mastuj Valley is a high altitude grazing land. Qualitative screening sometimes fails to mark the presence or absence of particular phytochemical due undetectable trace amount of material. We used one solvent and collected plants from one locality due to time, physical and financial limitations. This preliminary qualitative effort, therefore, can be advanced further towards quantitative analysis using more plant species from different locations, seasons and extraction with various solvents of different polarity to exactly quantify the phytochemicals at different growth stages in different parts of the plant.

Acknowledgement

The authors are obliged to Higher Education Commission, Islamabad for providing financial assistance under Project No. 20-352/R&D/05 (to FH). We are also thankful to the local community for providing local hospitality, accompanying in field work and supporting in difficult situation.

References

Abbas, A., S.A.R. Naqvi, M.H. Rasool, A. Noureen, M.S. Mubarik and R.B. Tareen. 2021. Phytochemical analysis, antioxidant and antimicrobial screening of *Seriphidium oliverianum* plant extracts. Dose-Response: *An Int. J.*, 2021:1-9. DOI: 10.1177/15593258211004739 journals.sagepub.com/home/dos

Abeysinghe, D.T., K.A.H. Kumara, K.A.D. Kaushalya, U.G. Chandrika and D.D.D.H. Alwis. 2021. Phytochemical screening, total polyphenol, flavonoid content, *In vitro* antioxidant and antibacterial activities of Sri Lankan varieties of *Murraya koenigii* and *Micromelum minutum* leaves. *Heliyon,* 7(2021): e07449.

Adegbaju, O.D., G.A. Otunola and A.J. Afolayan. 2020. Effects of growth stage and seasons on the phytochemical content and antioxidant activities of crude extracts of *Celosia argentea* L. *Heliyon,* 2:6(6): e04086. doi: 10.1016/j. heliyon. 2020. e04086. eCollection 2020 Jun.

Afzal, T., Y. Bibi, M. Ishaque, S. Masood, A. Qayyum, S. Nisa, Z. Hussain Shah, H. Alsamadany and G. Chung. 2022. Pharmacological properties and preliminary phytochemical analysis of *Pseudocaryopteris foetida* (D. Don) P. D. Cantino leaves. *Saudi J. Biol. Sci.*, 29: 1185- 1190.

Akinmoladun, A.C., O.E. Falaiye, O.B. Ojo, A. Adeoti, Z.A. Amoo and M.T. Olaleye. 2022. Effect of extraction technique, solvent polarity, and plant matrix on the antioxidant properties of *Chrysophyllum albidum* G. Don (African Star Apple). *Bull. Nat. Res. Cen.,* 46: 40. https://doi.org/10.1186/s42269-022-00718-y

Aljaiyash, A., A. Kasratic, H. Labiad, C.D. Alaouid, M. Ghanmie, B. Satranie and A. Chaouch. 2022. Effect of phenological stages on yield, chemical composition and biological properties of essential oil from *Thymus maroccanus* Ball. *Arab. J. Med. & Arom. Plants (AJMAP), V8N1* 2022189-208.

Balamurugan, V., S. Fatima and M.A.S. Velurajan. 2019. A Guide to Phytochemical analysis. *IJARIIE-ISSN (O)*-2395-4396. 5(1): 236-245.

Banu, K.S. and D.L. Cathrine. 2015. General Techniques involved in phytochemical analysis. *Internat. J. Adv. Res. Chem. Sci.,* 2(4):25-32.

Bashir, S.F. and G. Kumar. 2021. Preliminary phytochemical screening and *In vitro* antibacterial activity of *Plumbago indica* (Laalchitrak) root extracts against drug-resistant *Escherichia coli* and *Klebsiella pneumonia*. *De Gruyte Open Agri.*, 6: 435-444.

Choukri, H., K. Hejjaoui, A. El-Baouchi, N. El-haddad, A. Smouni, F. Maalouf, D. Thavarajah and S. Kumar. 2020. Heat and drought stress impact on phenology, grain yield, and nutritional quality of Lentil (*Lens culinaris* Medikus). *Front. Nutr.,* 7:596307. doi: 10.3389/fnut.2020.596307.

deSouza, L.B., A.L. Gindri, T. de A. Fortes, T.F. Kubiça, J. Enderle, R. Roehrs, S. Moura Silva, V. Manfredini and E.G. Denardin. 2022. Phytochemical analysis, antioxidant activity, antimicrobial activity, and cytotoxicity of *Chapt alianutans* leaves. *Adv. Pharmacol. & Pharm. Sci.*, 2020.Article ID 3260745, https://doi.org/10.1155/2020/3260745.

Elaigwu, M., H.O.A. Oluma, C.U. Aguoru and A. Onekutu. 2020. Screening and phytochemical analysis of some plants extracts against aflatoxin producing fungi in sesame, Benue State, Nigeria. *Amer. J. Plant Sci.*, 11: 344-353.https://doi.org/ 10.4236/ajps.2020.113025.

Farag, R.S., M.S. Abdel-Latif, H.H. El-Baky and L.S. Tawfeek. 2020. Phytochemical screening and antioxidant activity of some medicinal plants 'crude juices. *Biotech. Reports*, 28(2020): 1-7. e00536

Fazili1, M.A., I. Bashir, M. Ahmad, U. Yaqoob and S.N. Geelani. 2022. *In vitro* strategies for the enhancement of secondary metabolite production in plants: a review. *Bull. Nat. Res. Cent.*, 46: 35. https://doi.org/10.1186/s42269-022-00717-z

Feudjio, C´esaire, M.A. Yameen, G.S.S. Njateng, M.A. Khan, S.L. Tamekou, J. Deke, S. Mpetga and J. Kuiate. 2020. The influence of solvent, host, and phenological stage on the yield, chemical composition, and antidiabetic and antioxidant properties of *Phragmanthera capitata* (Sprengel) S. Balle. *Evidence-Based Complementary & Alternative Medicine,* Volume 2020, Article ID 6284925. https:// doi.org/10.1155/2020/6284925.

Gomes, M.A., B.E.A. de Magalhães, W.N.L. dos Santos and J.R.G. da Silva Almeida. 2022. Influence of seasonality on phytochemical composition, phenolic content and antioxidant activity of *Neoglaziovia variegata* (Bromeliaceae). *Biointerface Res. Appl. Chem.*, 12(3): 2889-2904. https://doi.org/10.33263/BRIAC123.28892904.

Gwari, A., A. Negi and M. Mishra. 2021. Pharmacognostic and preliminary phytochemical screening of *Ocimum sanctum* Linn. Stem (Holy Basil), A known Indian folk medicinal plant. *Int. J. Pharm.,* 11(8): 17-22.

Hussain, F., S.M. Shah and H. Sher. 2007. Traditional resource evaluation of some plants of Mastuj, District Chitral, Pakistan. *Pak. J. Bot*., 37(2): 339-354.

Ibrahim, M., N. Akhtar, Sara and H. Bahadar. 2021. Proximate nutritional, elemental and phytochemical analysis of selected wild edible plants of District Malakand, Pakistan. *Pure & Appl. Biol.*, 10(3):781-788. http://dx.doi.org/10.19045/bspab.2021.10008.

Iheagwam, P.N., E.N. Onyeike and B.A. Amadi. 2019. Phytochemical and vitamin contents of *Mangifera indica* (Mango) fruits subjected to ripening by artificial methods. *Int. J. Environ. Agric & Biotech.*, 4(3): 677-584.

Khan, T., M.A. Khan, K. Karam, N. Ullah, Z.U. Mashwani and A. Nadhman. 2021. Plant *In vitro* culture technologies; a promise into factories of secondary metabolites against COVID-19. *Front. Plant Sci.,* 15: 1-21.

Lydia, M. Clemen-Pascual, R.A.S. Macahig and N.R.L. Rojas. 2022. Comparative toxicity, phytochemistry and use of 53 Philippine medicinal plants. *Toxicol. Reports,* 9 (2022): 22-35.

Mansoori, A., N. Singh, S.K. Dubey, T.K. Thakur, N. Alkan, S.N. Das and A. Kumar. 2020. Phytochemical characterization and assessment of crude extracts from *Lantana camara* L. for antioxidant and antimicrobial activity. *Front. Agron*., 2:582268. doi: 10.3389/fagro.2020.582268.

Marami, L.M., G.M. Dilba, D.T. Babele, E.J. Sarba, A. Gizaw, W. M. Bune, M. D. Bayu, P. Admasu, A. Mekbeb, M. Tadesse, K. Abdisa and D. Bayisa. 2021. Phytochemical screening and *In vitro* evaluation of bacterial activities of *Echinops amplexicaulis*, *Ruta chalpensis* and *Salix subserrata* against selected pathogenic bacterial strains in west Shewa Zone Ethiopia. *J. Exp. Pharmacol.*, 13: 5110520.

Mehmood, S., F. Syed, M.I. Khan and R.M. Aadil. 2021. Bioactivity evaluation and phytochemical screening of *Euphorbia helioscopia* and *Rumex dentatus*. *Food Sci. & Technol.*, Campinas, v42, e66721, 2022. DOI: https://doi.org/10.1590/fst.66721.

Mohan, E, S. Suriya, S. Shanmugam and K. Rajendran. 2021. Qualitative phytochemical screening of selected medicinal plants. *J. Drug Deliv. & Therap.*, 11(2):141-144. DOI: http://dx.doi.org/10.22270/jddt.v11i2.4609.

Mokua, S.K., J.M. Mbaria, T. E. Maitho and G.A. Moriasi. 2021. Ethnobotanical documentation, phytochemical screening and cytotoxicity evaluation of medicinal plants used to manage snakebite envenomation in Mwingi West

Subcounty, Kenya. *Evidence-Based Complementary & Alternative Medicine,* 2021, Article ID 4167296, https://doi.org/10.1155/2021/4167296.

Mollaei, S., V. Lotfizadeha, D. Dastanhttps and P. Asgharian. 2020. Phytochemical variation and biological activities of *Zosima absinthifolia* during various stages of growth. *Int. J. Food Proper.*, 2020, 23(1):1556-1567. https://doi.org/ 10.1080/ 10942912.2020.1818778.

Nagano, M.S. and C. Batalini. 2021. Phytochemical screening, antioxidant activity and potential toxicity of *Azadirachta indica* A. Juss (Neem) leaves. *Rev. Colomb. Cienc. Quim, Farm.*, 50(1): 29-47.

Onefeli, A.O., O.C. Jegede and A.I. Tay. 2021. Influence of plant part and age on phytochemical constituents of Monkey Kola (*Cola millenii*K. Schum.); an important agroforestry species. 4: x. https://doi.org/10.3390/xxxxx

Otari, S.S., S.B. Patel, M.M. Lekhak and S.G. Ghane. 2022. Phytochemical studies on two unexplored endemic plants of India, *Barleria terminalis* and *Calacanthus grandiflorus*. *Front. in Pharmacol.*, 12: 817885. https://doi.org/10.3389/ fphar.2021.817885.

Pereira, J.I.A., F.A. Pereira, G.S. Araújo, J.M.A. Rodriguesm, R.E. de Sá, J.M.T. Souza, A.B. Barros, M.D.P. Brito, A.R. de Araújo, L.M.C. Véras, D.A. da Silva, J.D.B.M. Filho and A.J. Araúj. 2022. Phytochemical investigation and biological activities of extracts obtained from *Montrichardia linifera* (Arruda) Schott leaves collected in different localities from Piauí coast. *Res. Soc.&Develop.,* 11(4): e23011427231, 2022. (CC BY 4.0)| ISSN 2525-3409.DOI:http://dx.doi.org/ 10.33448/rsd-v11i4.27231.

Reaisi, R., M. Yadegari and H.A. Shirmardi. 2019. Effects of phenological stage and elevation on phytochemical characteristics of essential oil of *Teucrium polium* L. and *Teucrium orientale* L. *Int. J. Hort. Sci. & Technol.,* 6(1): 89-99. DOI: 10.22059/ijhst.2019.275327.276. https:// ijhst.ut.ac.ir, Email: ijhst@ut.ac.ir

Rubio, J., G. Arias, C. Robles-Kelly, E. Silva-Moreno, L. Espinoza, H. Carrasco and A.F. Olea. 2022. Phytochemical profiling and assessment of anticancer activity of *Leptocarpha rivularis* extracts obtained from *In vitro* cultures. *Plants*, 11:546. https://doi.org/ 10.3390/ plants11040546.

Shah, S.M. and F. Hussain. 2014. Evaluation of micro-minerals and nutritional status of some forage grasses in Mastuj Valley, Hindu Kush Range, Pakistan. *Pak. J. Nutr.*, 13 (11): 622-625.

Sharifi-Rad, M., P. Pohl, F. Epifano, G. Zengin, N. Jaradat and M. Messaoudi. 2022. *Teucrium polium* L.: Phytochemical screening and biological activities at different phenological stages. *Molecules*, 27: 1561. https://doi.org/10.3390/molecules27051561

Shukla, V., P. Singh, D. kumar, R. Konwar, B. Singh and B. Kumar. 2021. Phytochemical analysis of high value medicinal plant *Valeriana jatamansi* using LC-MS and it's *In-vitro* anti-proliferative screening. *Phytomed. Plus*, 1(2): 100025. https://doi.org/10.1016/j.phyplu.2021.100025.

Sreenivasulu, N. and A.R. Fernie. 2022. Diversity: current and prospective secondary metabolites for nutrition and medicine. *Curr. Opin. Biotech.,* 74: 164–170. www.sciencedirect.com

Sulaiman, S.M. Shah, Sadaf, M. Amin, B. Gul, and M. Begum. 2020. Ethnoecological, elemental, and phytochemical evaluation of five plant species of Lamiaceae in Peshawar, Pakistan. *Scientifica,* 2982934, https://doi.org/10.1155/ 2020/2982934.

Trease, G.E. and W.M. Evans. 2009. Pharmacognosy.16th Edition. Saunders Elsevier, London.

Twaij, B.M. and N. Md. Hasan. 2022. Bioactive secondary metabolites from plant sources: types, synthesis, and their therapeutic uses (Review). *Int. J. Plant Biol.,* 13: 4-14. https://doi.org/10.3390/ijpb13010003

Verdecía, D.M., R. del Carmen. Herrera-Herrera, E. Torres, A.R. Sánchez, L.G. Hernández-Montiel, R.S. Herrera, J.L. Ramírez, R. Bodas, F.J. Giráldez, J. Guillaume, H. Uvidia and S. López. 2021. Primary and secondary metabolites of six species of trees, shrubs and herbaceous legumes. *Cuban J. Agri. Sci.*, 55(1): 77-93.

Wawrosch, C., and S.B. Zotchev. 2021. Production of bioactive plant secondary metabolites through *In vitro* technologies status and outlook. *Appl. Microb. & Biotech.*, 105: 6649-6668. *https://doi.org/10.1007/s00253-021-11539-w*

Yu, M., I. Gouvinhas, J. Rocha and Ana I.R.N.A. Barros. 2021. Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources. *Sci. Reports,* 11:10041. https://doi.org/10.1038/ s41598-021-89437-4.

(Received for publication 27 July 2021)