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Research Article

An Updates on *Chrozophora Tinctoria* for Its Medicinal Values

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ABSTRACT

The present review work deals with the complete study of *Chrozophora tinctoria* for assess the description, cultivation, quality of nutrients, phytoconstituents, microbial effect and beneficial uses of the whole parts. The development of the roots stem and leaves of the plant were observed under various fertilizers, soil, lime and in watering condition at altitudes of 0-1650 meters and observed that the fertilizers inhibited growth and development of the root while stimulating the stems and leaves. The plant is medicinally used as cathartic, emetic, antipyretic, wart etc. and as a medieval colorant science the 17th century and also used as a source of dye for Turkish carpets and Palestine used much like henna to dye the fingernails. The regression analysis determines the relationships between the organic matter, P, K, pH, total soluble salts, $CaCO_3$ content of the soils and the N, P, K, Ca, Na, Mn, Zn and Cu content of the *C. tinctoria* plant. The methanol extract of the aerial parts possess four known flavonoid glycosides, i.e., quercetin 3-*O*-rutinoside, acacetin 7-*O*-rutinoside, apigenin 7-*O*-D-[(6-*p*-coumaroyl)]-glucopyranoside, and apigenin 7-*O*-D-glucopyranoside. One novel acylated flavonoid glucoside, viz. apigenin 7-*O*-D-[6-(3,4-dihydroxybenzoyl)]-glucopyranoside (chrozophorin) was recently isolated.

KEYWORDS

Chrozophora tinctoria, turnsole, quercetin, acacetin, apigenin, chrozophorin.

1. INTRODUCTION

Chrozophora tinctoria is an annual prostrate herb commonly known as dyer's-croton, giradol or turnsole. It is a flowering plant of genus *Chrozophora* belonging to family Euphorbiaceae contains hermaphrodite flowers and firstly described in 1824 comprising as monoecious under shrubs [1,2]. The genus of this plant is widespread across Europe, Africa, and Asia. *C. tinctoria* produces blue-purple stain colorant known as "turnsole" used in medieval enlighten documents and also as food colorant. The flowering and fruiting occurs in the month of April-July every year and common of dry waste places on sandy or loam clay. It can grow in light (sandy), medium (loamy) and heavy (clay) soils at acid pH, neutral or alkaline soils. The environmental condition required for its growth is semi-shade (light woodland) or no shade mostly prefers moist soil [3].

Fig. 1. *Chrozophora tinctoria* (L.) Raf plant.

1.1. Botanical description of the plant

Plant Structure: Characteristic growth form branching
Surface Description: Upright errect, well clear vertically straight up of the ground
Moderately Branched: Extensive number of secondary branches along the main stem
Stellate: Hairs radiated out from an ordinary point, like the points of a star
Leaves: Characteristic arrangement attachment venation
Description Alternate: Growing at different positions along the stem axis
Petiolate: Hanging out by a cylindrical leaf-stalk
Pinnate venation: Lateral veins diverged from the midrib towards the leaf margins
Leaf color: Ash-green easily spotted in its habitat
Flowers: Basic flower with number of petals and sepals possess characteristic color
Habitat: It occurs in seasonally inundated habitats such as rivers and run-off channels.

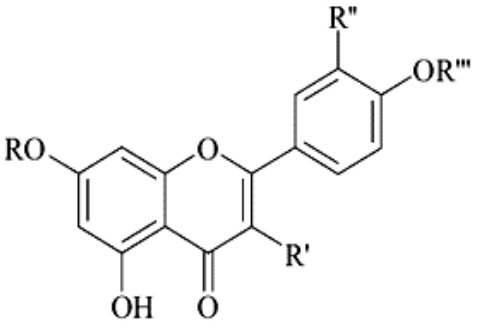
The plant has the ash-grey appearance because it is densely covered with white, wool-like hairs. The hair is stellate because of groups of hair bristle are arranged as radiating out from a common point and looks like the pointed star. The plant produces few simple branches started from one third up of the plant height with thin blast stem that yellow–amber in color. Leaves are developed alternatively along with the stem and found in very small numbers per plant. The

mature leaves have a long petiole and rhombic to ovate shape. The margins of leaves are sinusoidal with perpendicular plane to the lamina plane.

The *C. tinctoria* is a monoecious plant hence separately produces tiny male and female flowers, which become inconspicuous. The male flowers consist of 5 sepal calyx, 5 yellow petals and a cluster of 5 central stamens, which have dark or black in color. The female flowers have a 10 sepal calyx around the spherical ovary, no petals and 3 styles with each subdivided into 2 stigmas. The male and female flowers outgrow as a raceme at the top of the branch but they are so densely packed and appear to be as spiked. Male flowers are above the basal female flowers in the spike-like raceme and pollinated by a small-sized species of ants.

The fruit is looking a strange capsule with the shape of 3 spherical bodies fused in a rather rounded-triangular structure. Additionally, the fruit has perpendicular stubby projections and white scales contrasting with the dark green wall of the fruit holding 3 seeds. Fruits are the most conspicuous part of the plant, become dark green color at complete maturity and eventually burst open with an incredibly strong and sudden twist of its walls, sending the seeds inside to a considerable distance away. The seeds are oval or teeth shaped with the rough texture. They are 4 mm in size and grey to light brown color and the remnants of the fruit wall (found on the soil under the plant) rapidly turn black [4.5].

The parts of plant generally used for the medicinal purpose are leaves, roots, and fruits. The HPLC analysis of the methanol extract of the aerial parts of *Chrozophora tinctoria* yielded five flavonoid glycosides as active constituents, these were as follows; (1) quercetin 3-o-rutinoside (rutin), (2) acacetin 7-o-rutinoside, (3) apigenin 7-o-b-d-[(6-p-coumaroyl)]-glucopyranoside, (4) apigenin 7-o-b-d-glucopyranoside (5) apigenin 7-o-b-d-[6-(3,4-dihydroxybenzoyl)]-glucopyranoside (chrozophorin) the last one was reported as a new natural product [6].



Compound number	R	R'	R''	R'''
1	H	Rutinosyloxy	OH	H
2	Rutinosyl	H	H	Me
3	(6-*O*-*p*-coumaroyl)-glucosyl	H	H	H
4	Glucosyl	H	H	H
5	(6-*O*-3,4-dihydroxybenzoyl)-glucosyl	H	H	H

As a part of continuing search for natural antioxidants; the isolation, structure determination, and free radical scavenging activity of four known flavonoids (1-4) and a new acylated flavone glucoside, named chrozophorin (5), from the aerial parts of *C. tinctoria* [7,8].

1.2. Ecology

The species is native to a number of countries in Africa, (Algeria, Egypt, Libya, Morocco, Tunisia and Yemen) temperate and tropical Asia (Kuwait, Saudi Arabia, Afghanistan, Iran, Iraq, Israel, Jordan, Lebanon, Syria, Turkey, Kazakhstan, Turkmenistan, India and Pakistan), and Europe (Ukraine, Albania, Bulgaria, Greece, Italy, Malta, France, former Yugoslavia, Portugal and Spain) [9,10]. *Chrozophora tinctoria* is well known for producing dye substances and flavonoids. Alkaloids, coumarins, chromones, flavonoids, xanthones, diterpenoids and phenylpropanoid glycosides have previously been reported from a few other species of the genus *Chrozophora*. While, in the Iranian traditional medicine, used to treat warts, this plant has been used as emetics, cathartics, treatment of fever etc [11,12].

1.3. Epidemiology

The plant is herb or undershrub, monoecious, consisting very dense indumentum, stellates are sessile and peduncled, simple as well as lepidote hairs. Narrow triangular stipules, caduceus, and very indistinct scars. Leaves spirally arranged, simple petiole in transverse section: blade ovate, often 3-lobed, coriaceous, symmetric, margins shallowly to laxly but distinctly crenate, apex rounded, upper surface less densely covered by hairs than lower surface, the latter usually basally with 2 glands near insertion and often with submerged glands, glands crater-like, venation basally trinerved, pinnate along midrib with nerve ending open in the margins, veins laxly scalariform quaternary veins reticulate [13,14]. Inflorescences terminal racemes, the fruits are in pseudo-lateral and opposite to leaf due to the extension of axillary buds; solitary not branching, basal flowers pistillate, apical ones staminate, rachis tomentose [15].

The staminate flowers typically 2 per node and the pistillate flower generally single pedicels with abscission zone; calyx 5-lobed, valvate, tormentose outside, glabrous inside, petals, disc indistinct, blabrous, calyx campanulate, lobes ovate, petal slightly larger than sepals obovate petaloid margins in upper part undulate with simple hairs on both sides; disc divided into small glands, united with petals. Stamens, glabrous, filament united into a column branching off in 3 layers, free part thread like anthers basidorsi-fixed, 4 locular, opening extorse with lengthwise slits, pistillode absent. Pistillate flowers pedicellate, calyx and petals persistent, both 5-merous, narrowly ovate, hairy outside, glabrous inside, petals sepaloid in texture, slightly smaller than calyx, disc annular, very flat pistil 3–locular, on short gynophore, one ovule per locule, smooth, covered by flat stellate hairs, style short, hairy persistent stigma almost completely divided, below stellar hairy, above with long slender papillae, persistent. Fruits slightly lobed capsules, triangular in transverse section, dehiscing usually septicaidally and partly loculicidally into 3 bivalved parts, outside densely stellate, inside glabrous thin-walled, column slender, with frayed remnants of the septa, apically triangular; septa single veined. 3 seeds per fruit, obovate, angular covered by thin, incomplete sarcotesta, the latter carunculate, apically flat embryo, and copious endosperm.

1.4. Edible uses

The red and blue edible dyes are obtained from the flowers, fruit, and sap. It was used to give extra color to hippocras and other compounded wines with the seed of the small turnsole. It dye and stay old linen clothlets and rages into purple color where withal in this country, men use to color jellies, wines, and fine confections. Turnsole was considered as another kind of litmus and sometimes was used for coloring Dutch cheese and certain liquors.

1.5. Medicinal properties

This pant is reported to have the following medicinal properties;

Cathartic- Cleansing the bowels, promoting evacuations by stoop: purgative

Emetic- An agent that causes vomiting, so as the stomach is emptied of its contents

Fever- A medicine that lowers body temperature to prevent or alleviate fever-damage

Wart- Used to control and treat warts

1.6. Historical use as a colorant

Chrozophora tinctoria is a medieval colorant in the 17th century. The most organic colorant in medieval manuscript illuminations are the result of metalloorganic complexation where the organic substance is precipitated with a metal salt into an insoluble pigment lake. Sometimes miniature painters used very specific different colorants in a pure and uncomplexed form, known as clothlets. In Turkey, it has long been used as a source of dye for Turkish carpets and Palestine in used much like henna to dye the fingernails. It grows on dry fallow land amongst crops such as wheat or onions in vineyards [16].

Different botanical sources and their coloring component could be used for this purpose. The most important of these is *Chrozophora tinctoria* Juss. This grayish-green annual, belonging to the Euphorbiaceae, has found a description in several medieval technical treatises. It is generally thought that Chrozophora clothlets ceased to be used with the ascending of the book printing industry after 16th century [17].

The plant is cultivated in France, confined to a village named grand–gallargues in the neighborhood of Nimes. The greenish juice in contact with ammonical liquid yields a kind of litmus, which turns red by acids but does not become blue again under the influence of alkalies. Paint rags are made by dipping pieces of muslin into the juice and exposing them to the ammonical vapors arising from a mixture of urine and lime, or from, horse–dung until the desired color is produced. This material is slated to be mostly exported to Holland, where it is used for the coloring of cheese and of certain liquors [18].

Chrozophora tinctoria is a dye plant native to Syria used as an illuminators pigment and as a blue food coloring and commercially sold in Europe for staining rags. The term turnsole came to mean any such rag used as a coloring agent whether the original source of color was the herb turnsole. Turnsole is a purple dye stuff formerly much used as a food coloring especially for jellies. It is obtained from the fruits of *Chrozophora tinctoria*, a member of the spurge family native to the Mediterranean. The green juice of the fruits was pressed out with a roller and course linen rags were allowed to soak it up and dried on exposure to air or ammonia fumes, turned a beautiful purple color. Once the turnsole was much cultivated in the south of France but is rarely met with in modern times, due to the widespread use of synthetic dyes.

2. MATERIALS AND METHODS

2.1. Plant collection and authentication

The aerial parts of *Chrozophora tinctoria* were collected from Rajesultanpur, District Ambedkar Nagar, Utter Pradesh (India) during the month of May-June and the identity was confirmed by morphological characterization in comparison with the herbarium specimen retained in the Botanical Survey of India, Northern Regional Centre, 192 Kaulagarh Road, Dehradun. Also, a voucher specimen (Acc. No. 114546) representing this collection has been available in the herbarium department, BSI, Dehradun, Uttarakhand, India.

2.2. Toxicity report

Few reports such as those stated by the use food and drugs administration indicate that the plant has toxic properties to some farm animals and even to man. Furthermore, a document tilled sand dune vegetation of the Cholistan and some control measures against wind erosion written by Dr. Mirza Hakim Khan listed that *Chrozophora tinctoria* as an unpalatable poisonous plant and needs attention for its control [19].

2.3. Morphological study

The morphological characteristics of the root, stem, leaf, flower, seed, and fruits of *Chrozophora tinctria* Rafin. distributed in west Anatolia was determined, and biometric measurements were taken from collected samples of 42 different localities [20]. The development of the roots stem and leaves of the plant were observed when grown under various fertilizers, soil, lime and watering condition and their biometric measurement was taken. The plant did not show any sign of growth or development under shade and lime condition. Fertilizers inhibited growth and development of the root while stimulating the stems and leaves. The plant grows under various conditions at altitudes of 0-1650 meters [21].

2.4. Instrumental analysis

UV spectra were obtained in MeOH using a Hewlett-Packard 8453 UV-vis spectrometer. NMR spectra were recorded in CD_3OD on a Bruker 200 MHz NMR Spectrometer (200 MHz for ^{1}H and 50 MHz for ^{13}C) using residual solvent peak as the internal standard. ESIMS analyses were performed on a Finnigan MAT95 spectrometer. HPLC separation was performed in a Shimadzu HPLC system. A Shim-Pak ODS column 10 mm, 250 mm x 21.2 mm was used. Sep-Pak Vac 35 cc (10 g) C_{18} cartridge (Waters) was used for pre-HPLC fractionation [22].

2.5. Regression analysis of soil

Suleyman Baslar et al. 1999 were collected the soil samples from the localities above between the months of July and August. The soil samples were collected, after cleaning of the litter on the soil, put into polyethylene bags and taken immediately to the laboratory. They were left under laboratory conditions and air-dried. The dried soil samples were ground, passed through a 2mm sieve and subjected to analysis. The texture, pH, water holding capacity, total soluble salts, calcium carbonate and organic matter content determinations were made according to the methods detailed (Table 1) by Ozturk et al. 1997 [23,24].

An attempt was made to determine relationships between the organic matter, P, K, pH, total soluble salts, $CaCO_3$ content of the soils and the N, P, K, Ca, Na, Mn, Zn and Cu content of the *C. tinctoria* plants. For the regression analysis relevant data were observed between pH and K;

and K and Ca, and the former between organic matter and Mn, Na and organic matter (Table 1) [25].

Table 1: Regression analysis of soil organic matter & plant manganese content in *C. tinctoria*

Linear fit					
Summary of fit					
Re square		0.245518			
Root mean square error		16.966675			
Mean of response		65.95238			
Observations (or Sum wets)		42			
Analysis of variance					
Source	DF	Sum of square	Mean square	F ratio	
Model	1	3747.074	3747.07	13.0165	
Error	40	11514.831	287.87	Prob>F	
C Total	41	15261.905		0.0008	
Parameter estimates					
Term	Estimates	Std error	t Ratio	Prob>ltl	
Intercept	81.09974	4.94784	16.39	0.0000	
Soil Organic Matter %	-6.26232	1.73575	-3.61	0.0008	
Bivariate					
Variable	Mean	Std Dev	Correlation	Signif.	Number
Soil Organic Matter %	2.41881	1.526578	-0.4955	Prob	42
Plant Manganese %	65.95238	19.29356		0.0008	

2.6. Extraction and isolation process

Gulam Dastagir and Farrukh Hussain 2014 reported that the dried and ground-aerial parts of *C. tinctoria* (100 g) were Soxhlet-extracted, successively, with *n*-hexane, dichloromethane, and methanol (1.1 L each); the values are given in table 2. The plant material (whole) was air-dried at room temperature for five days and pulverized with mortar and pestle. Four hundred grams of the powdered plant material was defatted with petroleum ether (60–80°C) using continuous soxhlet extraction method to exhaustion [26]. The extract was then concentrated *in vacuo* and greenish-yellow viscous oil 15.0 g (3.75% w/w) was obtained. The result was comprehensively extracted by the same method using 95% ethanol in water and a greenish-brown gummy mass 66.0g (16.5% w/w) was obtained after concentration *in vacuo***.** About 35 g of the crude ethanolic extract was completely suspended in 250 ml of distilled water and then filtered. The resulting filtrate was then partitioned with ethyl acetate until the resulting organic layer was visibly clear, the ethyl acetate soluble portion was then concentrated *in vacuo* to generate a yellowish-brown mass 2.25 g (6.43% w/w); then the resulting aqueous layer partitioned with n-butanol until the resulting organic layer was visibly clear; the n-butanol soluble portion was also concentrated ***in vacuo*** to generate a dark-brown mass 10.01 g (28.6% w/w). The residual aqueous layer was finally concentrated *in vacuo* to yield a reddish-brown mass 14.18 g (40.5% w/w). The extracts were stored aseptically in a desiccator at room temperature until demanded [27,28].

Table 2: Effects of different concentrations of the methanolic extract and n-hexane extracts of *C. tinctoria* and its cytotoxicity

Extracts	Conc. of extract (µg/ml)	*Extractive values*	Cytotoxicity
Methanolic extract	10	28.33	47.22
	50	0.00	
	100	100.0	
	300	100.0	
	1000	100.0	
	Mean	**65.67**	
n-hexane extract	10	7.037	151.77
	50	23.06	
	100	100.0	
	300	48.13	
	1000	100.0	
	Mean	**55.65**	

2.7. DPPH Assay

2,2-Diphenyl-1-picrylhydrazyl (DPPH), molecular formula $C_{18}H_{12}N_5O_6$, was obtained from Fluka Chemie AG, Bucks. Quercetin was obtained from Avocado Research Chemicals Ltd, Shore Road, Heysham, Lancs. The method used by Takao et al. 1994 was adopted with suitable modifications to our particular circumstance. Phytotherapy Res. h 21, 615-21 DPPH (4 mg) was dissolved in MeOH (50 mL) to obtain a concentration of 80g/mL [29,30].

Qualitative assay: Test samples (MeOH extract) were applied on a TLC plate and sprayed with DPPH solution using an atomizer. It was allowed to develop for 30 min. The color changes (purple on white) were noted.

Quantitative assay: The MeOH extract, and test compounds 1-5 were dissolved in MeOH to obtain a concentration of 1.0 mg/mL. Dilutions were made to obtain concentrations of $5x10^{-1}$, $5x10^{-2}$, $5x10^{-3}$, $5x10^{-4}$, $5x10^{-5}$, $5x10^{-6}$ $5x10^{-7}$, $5x10^{-8}$, $5x10^{-9}$, $5x10^{-10}$ mg/mL. Diluted solutions (1.00 mL each) were mixed with DPPH (1.00 mL) and allowed to stand for 30 min for any reaction to occur. The UV absorbance was recorded at 517 nm. The experiment was performed in triplicate and the average absorption was noted for each concentration. The same procedure was followed for the positive standards (quercetin).

2.8. Phytochemical screening

Ghulam Dastagir et.al 2013 reported that the crude ethanolic extract and its partitioned extracts were screened phytochemically for the presence of its constituents utilizing standard methods of analyses (Table 3) in Trease G E and Evans W C, Pharmacognosy 2002 [31,32].

Table 3: Proximate composition of *Chrozophora tinctoria* (L.) during winter

Part	Moisture contents (%)	Ash content (%)	Protein contents (%)	Fat contents (%)	Fiber contents (%)	Carbohydrate (%)	Gross Energy (Kcal/g)
Roots	6.1	8.3	3.0	8.5	56.3	17.8	439.1
Stems	10.1	15.7	6.9	8.5	30.9	27.9	380.6
Leaves	9.7	16.0	10.5	13.0	6.7	44.1	393.2
Fruits	8.0	15.3	6.8	7.6	7.2	55.1	367.4
Means	8.45	13.8	6.8	9.4	25.27	36.2	395.0

Macronutrients of plant during summer [33]

Table 4: Macronutrients status of plant *Chrozophora tinctoria* during summer

Parts	Ca (mg/L)		K mg/L)		Mg (mg/L)		Na (mg/L)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Root	251.50	09.41	26.97	0.001	10.91	0.052	1.994	0.0165
Stem	250.70	10.99	26.96	0.001	11.29	0.166	1.952	0.0065
Leaf	091.88	00.70	27.07	0.002	09.08	0.0636	1.555	0.0108
Fruit	082.06	00.59	27.10	0.002	08.24	0.0446	1.568	0.0063
Average	169.04	05.42	27.03	0.002	09.88	0.0825	1.767	0.0102

Table 5: Cr,Cu, Fe and Mn concentration of plant *Chrozophora tinctoria* during summer

Parts	Cr (mg/L)		Cu (mg/L)		Fe (mg/L)		Mn (mg/L)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Root	0.0	0.0039	0.059	0.0022	3.549	0.0106	0.265	0.0037
Stem	0.0	0.0047	0.073	0.0036	3.852	0.0094	0.432	0.0038
Leaf	0.0	0.0241	0.060	0.0023	2.989	0.0258	0.130	0.0017
Fruit	0.0	0.0058	0.031	0.0023	1.819	0.0096	0.122	0.0014
Average	0.0	0.0096	0.056	0.0026	3.052	0.0139	0.237	0.0027

Table 6: Mo, Zn and Al contents of plant *Chrozophora tinctoria*

Parts	Mo (mg/L)		Zn (mg/L)		Al (mg/L)	
	Mean	SD	Mean	SD	Mean	SD
Root	0.00	0.0118	0.211	0.0022	0.0	0.1198
Stem	0.00	0.0149	0.232	0.0016	0.0	0.0867
Leaf	0.01	0.0048	0.232	0.0021	0.0	0.0679
Fruit	0.00	0.0161	0.231	0.0019	0.9	0.0580
Average	0.003	0.0119	0.227	0.0020	0.23	0.0831

Macronutrients of the plant during winter [33]

Table 7: Macronutrients of plant *Chrozophora tinctoria* during winter

Parts	Ca (mg/L)		k (mg/L)		Mg (mg/L)		Na (mg/L)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Root	91.27	0.617	27.15	0.001	9.780	0.0987	8.740	0.1823
Stem	201.7	15.08	27.17	0.001	9.463	0.1177	8.375	0.1560
Leaf	221.8	03.23	27.14	0.001	9.101	0.0410	9.255	0.9327
Fruit	251.6	08.42	27.09	0.000	10.90	0.2480	8.148	0.0282
Average	191.593	6.837	27.138	0.001	9.811	0.126	8.6295	0.325

Table 8: Cu, Cr, Fe, and Mn contents of plant *Chrozophora tinctoria* during winter

Parts	Ca (mg/L)		k (mg/L)		Mg (mg/L)		Na (mg/L)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Root	0.0	0.0091	0.100	0.0019	4.866	0.0243	0.226	0.0088
Stem	0.0	0.0004	27.17	0.0038	2.643	0.0124	0.177	0.0023
Leaf	0.0	0.0158	27.14	0.0024	4.167	0.0100	0.196	0.0054
Fruit	0.0	0.0125	27.09	0.0025	6.027	0.0215	0.411	0.0004
Average	0.00	0.0095	20.375	0.0027	4.426	0.0171	0.2525	0.0042

Table 9: Mo, Zn, and Al contents of plant *Chrozophora tinctoria* during winter

Parts	Mo (mg/L)		Zn (mg/L)		Al (mg/L)	
	Mean	SD	Mean	SD	Mean	SD
Root	0.038	0.0026	0.160	0.0045	07.674	0.4596
Stem	0.063	0.0076	0.120	0.0012	08.968	0.4311
Leaf	0.022	0.0019	0.188	0.0037	11.830	0.3750
Fruit	0.022	0.0060	0.485	0.0065	00.812	0.5840
Average	0.036	0.0045	0.238	0.0040	07.321	0.4624

Seasonal variation of macro and micronutrients of the plant [33]

Table 10: Statistical analysis of seasonal variation of macronutrients and micronutrients of plant *Chrozophora tinctoria*

Macronutrients & Micronutrients	Summer	Winter	Mean
Ca (mg/L)	I69.00	191.60	180.30
K (mg/L)	27.00	27.14	27.07
Mg (mg/L)	09.90	09.81	09.85
Nr (mg/L)	01.77	08.63	05.20

Co (mg/L)	0.00	0.00	0.00
Cu (mg/L)	0.05	0.10	0.08
Fe (mg/L)	3.05	4.42	3.74
Mn (mg/L)	0.24	0.25	0.25
Mo (mg/L)	0.00	0.02	0.01
Zn (mg/L)	0.22	0.64	0.43
Al (mg/L)	0.90	7.21	4.05

Table 11: Variation in micro and micronutrients in two seasons of *Chrozophora tinctoria*

Season	Macronutrient (mg/L)				Micronutrient (mg/L)						
	Ca	K	Mg	Na	Al	Cr	Cu	Fe	Mn	Mo	Zn
Summer	169.0	27.00	9.88	1.76	0.16	0.0	0.05	3.05	0.24	0.0	0.22
Winter	191.6	27.14	9.81	8.63	7.32	0.0	0.09	4.43	0.25	0.02	0.26
Mean	180.3	27.07	9.85	5.20	3.74	0.0	0.07	3.70	0.25	0.02	0.24

Bacterial and fungal strains

The Gram-positive organisms used in this study are Bacillus subtilis (NCTC 8326 B76) and Staphylococcus aureus (ATCC 021001). Gram-negative organisms are Escherichia coli (ATCC 11775), Pseudomonas euroginosa (ATCC 10145) and Salmonella typhi. The fungal strains, Candida albicans, Aspergillus flavus and Aspergillus niger, are clinically isolated [34,35].

Table 12: Antibacterial activity of plant *Chrozophora tinctoria*

Plant extract	**Bacteria**	**Zone of inhibition (mm)**	**Percentage inhibition**	**Mean±SD (n=5)**
Methanol	Salmonella typhi	15	54.5	46.00±
	Pseud. aeruginosa	10	69.6	16.07
	Escherichia coli	19	42.4	
	S. aureus	25	24.2	
	Klebsiella pneumoniae	20	39.3	
n-Hexane	Salmonella typhi	18	45.4	41.16±
	Pseud. aeruginosa	24	27.2	22.25
	Escherichia coli	30	9.09	
	S. aureus	10	69.6	
	Klebsiella pneumoniae	15	54.5	

3. RESULTS AND DISCUSSION

RP-HPLC analysis of the methanol extract of the aerial parts of *C. tinctoria* afforded four known flavonoid glycosides, quercetin 3-*O*-rutinoside (1), acacetin 7-*O*-rutinoside (2), apigenin 7-*O*-D-

[(6-*p*-coumaroyl)]-glucopyranoside (3), and apigenin 7-*O*-D-glucopyranoside (4), and a novel acylated flavonoid glucoside, apigenin 7-*O*-D-[6-(3,4-dihydroxybenzoyl)]-glucopyranoside (named, chrozophorin, 5). The known flavonoid glycosides were readily identified by direct comparison of the data available from spectroscopic study [36]. The structure of the new compound was elucidated on the basis of comprehensive spectroscopic analyses (e.g. UV, ESIMS, and 1D and 2D NMR).

Flavonoids 1, 3 and 4 have previously been reported, in trace amounts, from the aerial parts of *C. tinctoria*. This is the first report on the occurrence of acacetin 7-*O*-rutinoside (2) in this species, and flavonoids 5 is a novel natural product. It is noteworthy that among the flavonoids (1-5) isolated in this study, flavonoids 2-5 are apigenin derivatives with glycosylation at C-7. The flavonoids found in this genus do not have oxygenation at C-3; while the family Euphorbiaceae is well known for producing various alkaloids, the distribution of flavonoids is rather limited to a few genera.

Morphologically, the male and female flowers are very small (1mm) and so inconspicuous. The male flowers have 5 yellow petals and a cluster of 5 black anthers at the center. The female flowers have no petals, only a globular ovary (enclosed by 10 sepals) with 3 yellow styles. The seeds available per fruit are 3 ovoid to teeth shaped and the shape is more or less oval but cross-sectional circumference has angular edges. The US FDA indicates that the plant has toxic properties to some farm animals and even to man, hence known as an unpalatable poisonous plant and needs attention for its control.

The crude MeOH extract showed low levels of free radical scavenging activity (antioxidant activity) (RC_{50} = 2.24 x 10^{-1} mg/mL) in the DPPH assay natural phenolic antioxidants, is a consequence of the presence of the phenolic moieties in the structures. The antioxidant activity of phenolic natural products is predominantly owing to their redox properties, i.e. the ability to act as reducing agents, hydrogen donors and singlet oxygen quenchers, and to some extent, could also be due to their metal chelation potential [37, 38].

4. CONCLUSION

Chrozophora tinctoria belonging to the family Euphorbiaceae is a monoecious widespread across Europe, Africa, and Asia highly cultivated for blue-purple stain colorant known as "turnsole" used in medieval enlighten documents and also as food colorant. The red and blue edible dyes are obtained from the flowers, fruit, and sap; while the turnsole considered as another kind of litmus and sometimes was used for coloring Dutch cheese and certain liquors. The environmental condition required for its growth is semi-shade or no-shade mostly prefers moist soil. It possess five flavonoid glycosides as active constituents, i.e., (1) quercetin 3-o-rutinoside (rutin), (2) acacetin 7-o-rutinoside, (3) apigenin 7-o-b-d-[(6-p-coumaroyl)]-glucopyranoside, (4) apigenin 7-o-b-d-glucopyranoside (5) apigenin 7-o-b-d-[6-(3,4-dihydroxybenzoyl)]-glucopyranoside (chrozophorin) the last one was reported as a new natural product. Medicinally the plant is used as cathartic, emetic, antipyretic and in case of wart [39,40].

The regression analysis relevant data were observed between pH and K; and K and Ca, and the former between organic matter and Mn, Na and organic matter. Qualitative and quantitative assay in MeOH extract of *C. tinctoria* reported the presence of phytoconstituents and trace

elements as micro and macro nutrients in different part of the plant such as, root, stem, leaf and fruit during summer and winter. The in-vitro antimicrobial activity was also reported against Gram-positive organisms like, *Bacillus subtilis* & *Staphylococcus aureus*, and Gram-negative organisms i.e., *Escherichia coli, Pseudomonas euroginosa* & *Salmonella typhi*. The fungal strains like, *Candida albicans, Aspergillus flavus* & *Aspergillus niger*.

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