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Research Paper

Studies on the pharmacognostical and *in vitro* antioxidant potential of *Cleome gynandra* Linn. leaves

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Abstract

***Cleome gynandra* Linn.** of Cleomaceae (Capparaceae) family is an annual herb, growing up to 0.6-1m in height and is popular in the Ayurveda, Siddha, Folk and Tibetian systems of medicine. The present paper deals with the pharmacognostical study, including the macroscopic, microscopic, fluorescence and phytochemical characteristics, which enables the identification of the leafy vegetable, as well as the determination of the antioxidant potential and the predominant classes of antioxidants that contribute to the activity.

Keywords: Antioxidant, *Cleome gynandra*, Flavonoids, Microscopic characters, Pharmacognostic characterization, Phytochemicals, Polyphenols, Trace elements, Vitamin C.

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Introduction

***Cleome gynandra* Linn.** [syn. *C. pentaphylla* Linn., *Gynandropsis pentaphylla* DC., *G. gynandra* (Linn.) Briq.] of Cleomaceae (Capparaceae) family is an erect glandular-pubescent annual herb, popularly used in the Ayurveda, Siddha, Folk and Tibetian systems of medicine. It is known as Cat's whiskers and Spider flower in English; *Cararvella, varvar, surjavarta* and *arkapushpika* in Sanskrit; *Arkahuli, karaila, hulul* and *churota* in Hindi; and *Velai keerai, neivayalla keerai* and *katte kadugu* in Tamil. This wild leafy vegetable is indigenous to the tropical and pan tropical regions and plays an important role in agricultural and nutritional systems of these regions[1-4]. In many cultures, the boiled leaves are regarded as medicinal meal for the treatment of various ailments. Bruised leaves are reported to be rubefacient, vesicant, antiseptic, anti-inflammatory and analgesic and hence used to treat local pains, neuralgia, rheumatism and scorpion-sting. Oral administration of a decoction or an infusion of the boiled leaves or the leaf-juice has been recorded to facilitate child birth, to relieve stomach pain, beneficial in constipation, thread-worm infection, conjunctivitis, oral ailments, convulsions and in certain bilious disorders[2-10]. Earlier investigations on the leaves of the Egyptian taxon have afforded certain flavonoids, triterpenoid saponins, sterols and fatty acids[11], a triterpene from the whole plant[12], glucosinolates[13] and a number of anti-tick essential oil constituents[14]. Extracts of the leaves and certain isolated flavonoids have been reported to possess antibacterial, antifungal, antineoplastic and anti-arthritic properties and improved the levels of endogenous antioxidants and also modulated glucose metabolizing enzyme activity[15-19].

Dietary phytochemicals, especially the polyphenolic antioxidants such as the ubiquitous flavonoids[20], polyunsaturated fatty acids, tocopherols, vitamin C and various inorganic micronutrients have been the subject of extensive research for their potential benefits to reduce the risk of degenerative diseases such as cardiovascular disease, several types of cancer, inflammation and neurological and other age-related disorders. Being diet derived, these compounds are generally regarded as safe chemicals based on their long history of use in the diet and have been demonstrated to possess strong antioxidant activities *in vitro*. Though the screening of antioxidant and radical scavenging activities of the taxon have been reported earlier[21, 22], but an investigation of the amounts of the potentially beneficial antioxidants available in the extracts of varying polarities of this leafy vegetable will also be of importance.

The plant grows wild, mixed with several other simulating weeds, viz. ***Cleome viscosa* Linn.**, ***Acalypha indica* Linn.**, ***Crotalaria verrucosa* Linn.**, ***Phyllanthus amarus* Schum. & Thonn.** and ***Heliotropium indicum* Linn.** In the absence of flowers,

distinguishing this medicinal herb from co-existing weeds is difficult. Therefore, studies on pharmacognostic characters of this plant would provide an account on correct identification of it.

Materials and Methods

Fresh leaf material was collected from their natural habitat in the suburbs of Puducherry and processed after preserving a voucher specimen in the herbarium of this Centre. Clearing of leaves was done to study venation pattern and microphotographs were taken with Canon camera fitted on binocular research microscope. The fluorescence and physico-chemical characters were determined as per standard methods. Powdered leaf material was successively extracted with n-hexane, benzene, chloroform, acetone and 90% ethanol using Soxhlet's apparatus and subjected to qualitative and quantitative analysis. Total content of the antioxidant principles and their activities were ascertained by extraction with 70% ethanol. Polyphenol and flavonoid contents of each extract (except the less significant, deeply coloured n-hexane, benzene and chloroform fractions) were determined[23, 24] using Shimadzu UV-160 Spectrophotometer and were expressed, respectively, as mg gallic acid equivalents and mg quercetin equivalents/100g fresh leaves. The amount of vitamin C in each extract was determined by redox titration and expressed as mg L-ascorbic acid/100 g fresh leaves. All values are expressed as mean ± SD of three replications. The antioxidant activities of the extracts were determined by the Vitamin C equivalent antioxidant capacity (VCEAC) assay and expressed as mg vitamin C equivalents/100 g of three replicates (mean ± SD). The analysis of the essential trace metal composition was carried out using fresh leaf material. The amount of Se and V were determined using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS), Agilent 7500 series and those of the others using Perkin-Elmer, Optima 2100 DV Optical Emission Spectrometer.

Results and Discussion

C. gynandra (Fig.1a) is an annual erect herb; 30-50cm in height. Long tap root with a few secondary shallow roots, stem unbranched to sparsely branched, glandular pubescent, but not spiny. Leaves alternate, digitately palmate, compound with 3 to 7 leaflets (Fig.1b), sessile, exstipulate; petiole 2-10 cm long, glandular; leaflets: obovate to elliptical or lanceolate 2-10 cm × 1-4 cm, cuneate at base, rounded to obtuse, acute or acuminate at apex, margins finely toothed, sparsely distinctly hairy. Leaves exhibit unicostate reticulate venation (Fig.1c). Primary veins straight, branched, divergence angle, secondary veins are thin, well developed areoles. Flowers bisexual, bracteate, white or tinged with purple; fruit long, cylindrical capsule, usually green or yellow, many-seeded and seeds sub-globose, grey to black, and irregularly ribbed. Entire plant emits a characteristic smell and tastes bitter.

Microscopic characters

Leaf thickness ranges from 112-398 µm. Upper epidermis single layered, large, slightly deep, tubular cell contains thick lamellar cuticle. Multiseriate glandular hairs embedded in both surfaces, foot 2-3 celled embedded in epidermis, bi-celled stalk, large columnar, head is about 3-5 tiered clavate. Mesophyll consists of palisade and spongy parenchyma; palisade cells are adaxial hypodermal, single layered, long rectangular with little inter cellular spaces, chloroplast abundant: spongy parenchyma 2-3 layered with large, intercellular spaces.

Vascular bundles large, collateral and arc-shaped in primary veins, small and round in secondary veins. Xylem towards adaxial side, phloem in abaxial, bundle sheath large, parenchymatous cells distinct, barrel shaped, bundles of tertiary veins buried between mesophyll cells. Lower epidermis single layered, large, thick walled; guard cells large, thick walled, vertically embedded to subsidiary cells thick-cuticle, lamellar, forming very minute outer ledges over guard cells.

a)Epidermis in surface view — The costal epidermal cells are large axially oriented 5-10 times longer than broad, rectangular to rhomboidal in shape, thick walled and straight. Intercostal cells are large and in variously shaped, thin walled, slightly to deeply sinuous. Evidently, three types of glandular hairs, namely uniseriate clavate, multiseriate-spherical and multiseriate-clavate were found in both costal and intercostal regions. Large shaggy glandular hairs infrequently distributed in intercostal region. Stomata distributed in costal and inter costal region, facing in all directions lying at level of epidermis; thickened at one or both the poles, medium sized, sub-spherical or elliptical stomatal dimorphism distinct. Among the three types, viz. anomocytic, anisocytic (Fig.1d) and tetracytic the third type is more frequently distributed. These three types of stomata are common in both the

Table 1: Microscopical characters of *Cleome gynandra* leaf

S. No.	Variables	Abaxial surface	Adaxial surface
1	Epidermal number /sq.mm	605.72	654.72
2	Stomatal number /sq.mm	164.92	174.84
3	Stomatal index /sq.mm	21.57	20.9
4	Stomatal size /µm	L: 29,B: 20.5	L: 24.42, B: 18.21
5	Epidermal cell size /µm	L: 70.29, B: 40.92	L: 24.42, B: 18.21

Palaside ratio: 4.2 - 6/sq.mm, Vein islet number: 91.76/sq.mm,
Vein termination number: 116/sq.mm

Table 2: Histochemical colour reactions of *Cleome gynandra* leaves

S. No.	Class of phytochemicals	Degree of the expected colour
1	Alkaloids	–
2	Lignin	++
3	Lipid & Lipoprotein	++
4	Mucilage	+
5	Proteins	++
6	Starch	+
7	Tannin	++

++ = Marked change, + = Moderate change, – = No expected change

Table 3: Fluorescence analysis of *Cleome gynandra* leaves

S. No.	Treatment for leaf powder	Under ordinary light	Under UV light (365 nm)
1	Powder as such	Dark green	Greyish
2	Powder + 1N HCl	Brown	Light green
3	Powder + 50%H_2SO_4	Dark green	Black
4	Powder + 10% NaOH (aqueous)	Dark brown	Dark yellow
5	Powder + 10% NaOH (ethanolic)	Dark green	Red
6	**Extracts**		
	a) n-Hexane	Dark yellow	Reddish yellow
	b) Benzene	Greenish yellow	Dark red
	c) Chloroform	Dark green	Dark red
	d) Acetone	Pale green	Pink
	e) Alcohol	Light yellow	Yellow
	f) Water	Light yellow	Pale pink

surfaces. Stomatal abnormalities such as those with aborted guard cells with thickened poles, juxtaposed contiguous stomata and shriveled stomata are rarely occurred. Stomata are 24-29 µm long and 18.21 to 20.5 µm broad. Stomatal index in abaxial surface was 21.57 µm and 20.9 µm in adaxial side. The epidermal cells on the adaxial (Fig.1e) sides are straite walled and abaxial epidermal cells are more undulated. Vein islet is distinct and is rhomboidal or broadly rectangular (Fig.1d). The average vein islet numbers are 91.76/sq.mm and vein termination number is 116/sq.mm (Table 1). The trichomes occur along the margins of the veins; they are either uni- or multi-seriate glandular hairs, some of them as shaggy glandular hairs (Fig.1f). The size of the epidermal cells and of stomata is greater in abaxial side while the number of stomata and epidermal cells are higher in adaxial as that of abaxial side (Table 1).

b) Histochemical analysis — The histochemical colour reactions of the leafy vegetable were performed as per Johansen[25]. Marked changes in colour were observed indicating the presence of lignin, lipoproteins, proteins and tannins (Table 2).

Fluorescence analysis

The powdered leaf was extracted with solvents of increasing polarity, viz. n-hexane, benzene, chloroform, acetone, alcohol and water successively and the fluorescence of each of these fractions as well as the extracts of the leaf drug in 1N HCl, 50% H_2SO_4 10% NaOH (aqueous) and 10% NaOH (ethanolic) were observed in day light and also under UV light (365nm) and recorded in Table 3.

Preliminary phytochemical analysis of the leaves

Preliminary phytochemical screening of the powdered leaf drug revealed the presence of carotenoids, cardiac glycosides, cyanogenetic glycosides, flavonoides and phenols, saponins, sugars, tannins and triterpenes and the absence of alkaloids and anthroquinone, as indicated in Table 4.

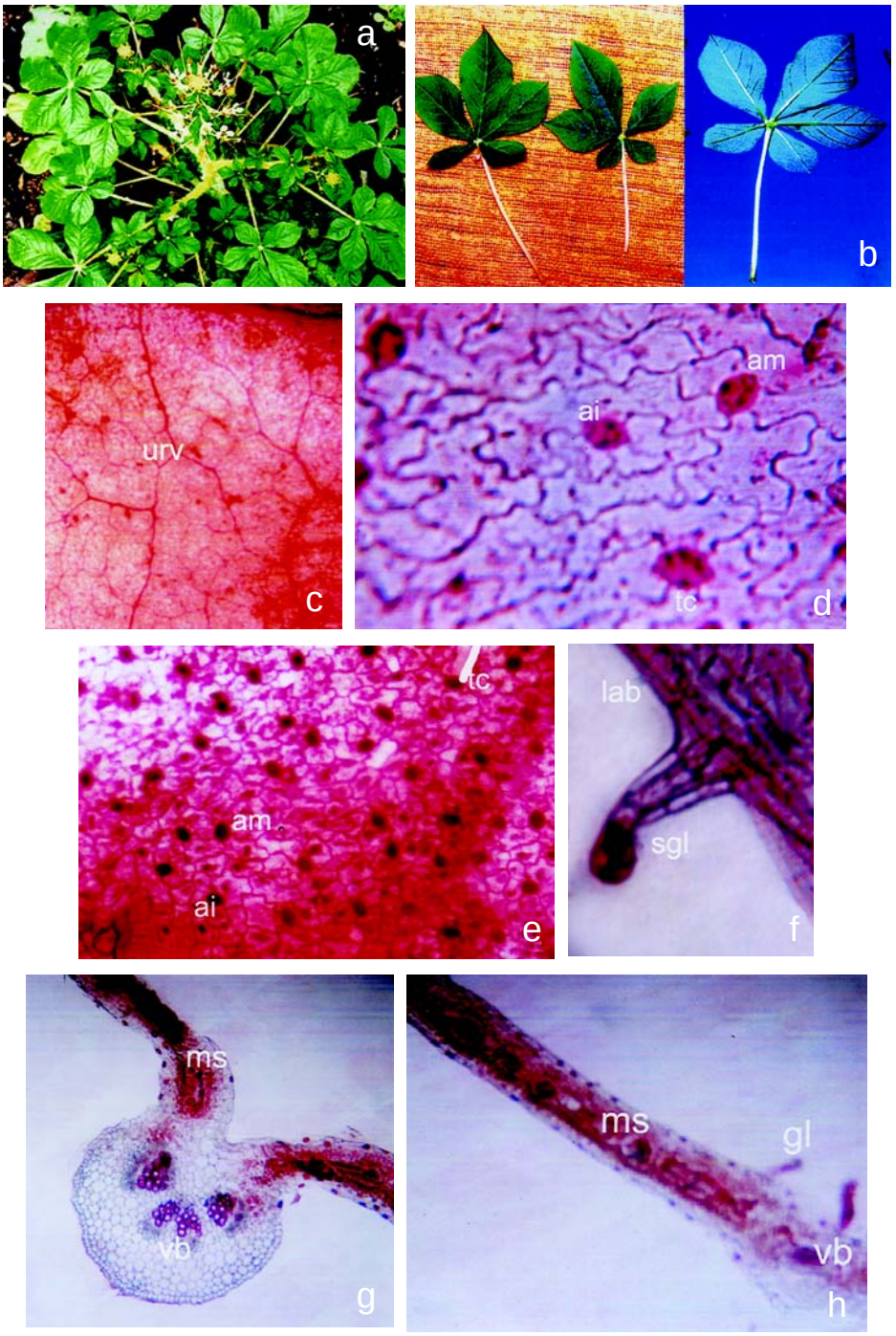
Salient features for the identification of crude drug

The crude drug of ***C. gynandra*** can be identified based on the following histo-morphological parameters, as per WHO guidelines for authentication of the drug, viz. the number of epidermal cells, morphology, leaf thickness, kinds of stomata and stomatal indices[26, 27], trichome and idioblasts frequency and size[28], bundle sheath characters (Fig.1 g), vein islets and palisade cells ratio (Fig.1 h).

The relevant preliminary histochemical analysis, phytochemical screens such as fluorescence analysis, phytochemical colour reactions, physico-chemical standards and isolation and identification of phenolic metabolites are additional corroborating parameters that could be applied successfully in the determination of the authenticity of the material.

Antioxidant activity

The total antioxidant activity, as evaluated by determining the ability of the leaf extract (70% ethanol) to scavenge the stable 2,2'-azinobis (3-ethylbenzothiazoline)-6-sulphonate radical cationic chromophore ($ABTS^{\cdot+}$), is found to be 523.67± 4.16 mg vitamin C



Figs. 1a-h: *Cleome gynandra*, (a) A portion of the plant with inflorescence (Top view); (b) Digitate leaves showing adaxial and abaxial surface; (c)Vein islet (10×40 µm); (d) Leaf abaxial (10×10 µm) showing highly sinuate walls and types of stomata; (e) Leaf adaxial (10×10 µm) surface showing slightly angular epidermal cells and kinds of stomata; (f) Leaf abaxial with shaggy glandular hair; (g) T.S. view of leaf on mid-rib; (h) T.S. of leaf lamina. (urv-unicostate reticulate venation, ai-anisocytic, am-anomocytic, tc-tetracytic stomata, lab-leaf abaxial, sgl-shaggy glandular hairs, ms-mesophyll, vb-vascular bundle, gl-glandular hair).

Table 4: Phytochemical analysis of *Cleome gynandra* leaves

Phytochemicals	n-Hexane	Benzene	Chloroform	Acetone	Ethanol (90%)	Water
Alkaloids	–	–	–	–	–	–
Anthraquinons	–	–	–	–	–	–
Carotenoids	–	–	+	+	–	–
Cardiac glycosides	–	–	+	+	++	++
Cyanogenetic glycosides	–	–	+	+	++	++
Flavonoids	–	–	–	++	++	++
Phenols	–	–	–	++	++	++
Saponins	–	–	–	++	++	++
Sugars	+	++	++	–	–	–
Tannins	–	–	+	+	++	++
Triterpenes	–	–	+	+	++	++

++ = Marked change, + = Moderate change, – = No characteristic change

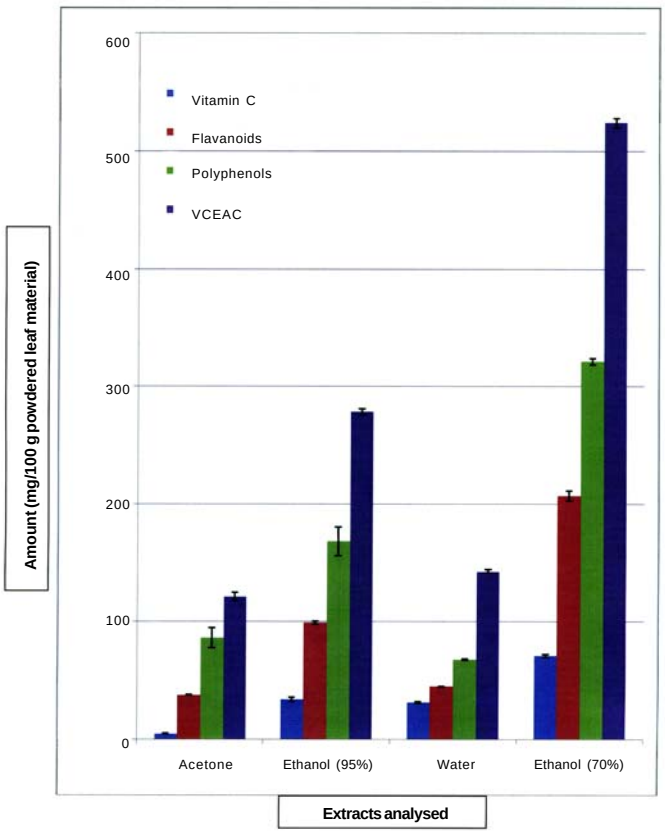


Fig. 2: Antioxidant activities of various extracts of *Cleome gynandra* leaves and contributing classes of compounds

equivalents/100 g (Fig. 2). Over the past two decades, an expanding body of evidences from epidemiological and laboratory studies have demonstrated that some edible plants as a whole, or their identified ingredients with potent antioxidant properties, especially the predominant polyphenolics, have substantial protective effects on human carcinogenesis, cardiovascular and renal disorders, memory and cognitive function, age-related neurological dysfunction such as Alzheimer's disease, diabetes, ulcers and several other human ailments[29-34]. Consequently, the contributions of the predominant classes of phytochemicals that are well recognised as potent antioxidants, viz. the polyphenols, in general, and the flavonoids, specifically, together with vitamin C, to the total radical scavenging potential have been evaluated. As can be observed from Fig. 2 polyphenols are the major contributors, amounting to 321.00 ± 2.65 mg gallic acid equivalents/100 g, out of which, flavonoids accounted for 207.00 ± 4.58 mg quercetin equivalents/ 100 g powdered leaf material. Vitamin C also contributed to a fairly significant amount of 70.67 ± 1.17 mg L-ascorbic acid equivalents/100 g to the total activity of the leaf extract. The distribution of these antioxidant biomolecules in various analysable fractions of the leaf powder extract, viz., the acetone, 90% ethanol and water soluble, as well as the relative polyphenolic, flavonoid and vitamin C contents in each of these fractions have also been determined and summarized in Fig. 2.

Though the antioxidant potentials of folk plants are largely attributed to certain classes of low molecular weight

secondary plant metabolites, they may also be influenced by certain essential and trace antioxidant micronutrients. In fact, studies have shown that additive and synergistic combinations of scores of phytochemicals and trace elements, which are either directly or indirectly involved in various redox processes are responsible for the observed health benefits of fruits and vegetables. Around 30% of enzymes and several other biomecules contain a trace metal at the active site and thus play a vital role in human metabolism. Micronutrient deficiencies affect nearly half the world's population, impairing child development, reducing work productivity, and increasing mortality and morbidity rates. All essential elements are primarily supplied through diet and therefore determination of the elemental composition of the food stuff and evaluation of their daily dietary intake becomes a necessity. Indian Council of Medical Research (ICMR) has recommended selective studies of individual foodstuffs as an important step in the estimation of dietary intake of trace elements[35]. Consequently, the results of the determination of certain essential trace metal composition are also summarized in Table 5.

Table 5: Essential mineral content of *Cleome gynandra* leaves

Analysis	Contents† (mg /100 g)
Moisture content	29.5258 ± 2.4491
Total ash	4.0939 ± 0.2632
Water insoluble ash	0.2264 ± 0.0362
Water soluble ash	3.8676 ± 0.2804
Magnesium	406.9333 ± 16.6182
Calcium	203.5000 ± 8.5294
Iron	11.9333 ± 1.3317
Chromium	5.2667 ± 1.1240
Copper	12.8000 ± 2.5515
Manganese	113.9333 ± 11.8006
Selenium	14.8000 ± 2.2913
Vanadium	7.6333 ± 0.8737
Zinc	30.3000 ± 1.7349

† Mean ± SD of three measurements

Conclusion

The results of the pharmacognostical characters of the leaves of ***C. gynandra*** such as the microscopic features may help in laying down micro-morphological standards as per WHO guidelines for authentication of the leaf drug. Adulterants, if any, can be easily identified using these parameters. Further, this study also implies that dietary polyphenolic phytochemicals, especially the flavonoids, vitamin C and essential metal ions accumulated in leaves may supply substantial antioxidants, which in turn, may inhibit, prevent or retard the development of several chronic diseases and thereby provide health-promoting effects.

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