ijlpr 2022; doi 10.22376/ijpbs/lpr.2022.12.6.L36-46

International Journal of Life science and Pharma Research

ISSN 2250-0480

Research Article

Pharmacognosy of Cleome Gynandra

Pharmacognostic and Phytochemical Features of *Cleome gynandra* Whole Plant and its Powder

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Abstract: *Cleome gynandra* is one among the plants used as a remedy for various human and animal diseases. It is commonly called nalvelai in Tamil and belongs to the family cleomaceae. Aim of the present study is used to assess quality of the powdered plant materials. The prime objective of this study is to assess characteristic features of powder preparation, pharmacognostic value like ash value, extractive value, chromophore by fluorescent assay and also to study phytochemical markers. Powder microscopy, organoleptic character assessment, physiochemical, fluorescent features, microbial load and preliminary phytochemical features like pharmacognosy of this plant were studied by making use of standard textual procedures. Plant powder is yellowish green in colour with a bitter taste. Plant powder showed 0% foreign matter, 4.9%w/w total ash with higher water extractive (23.7%w/w). Variable chromophores were detected with different acid and alkali treatment. Treatment of nitric acid showed violet brown coloration under long UV radiation. Microbial load was within the limits of pharmacopeial standards of raw drugs and no pathogenic organisms were detected. All the pharmacognostic results were within the limits of ayurvedic pharmacopeia of India. Whole plant powder showed the presence of terpenoids, flavonoids, polyphenols and saponins, which could be a phytochemical marker. GC-MS report revealed the presence of 20 Compounds in crude powder preparation. Histochemical study also confirmed the presence of tannin, saponin, flavonoids, steroids, Terpenoids and polyphenols, which could also confirm the water-soluble compounds in the *C. gynandra* whole plant powder. These observations would be of immense value in the botanical identification and standardization of drugs in a crude form.

Keywords: *Cleome gynandra*, Pharmacognosy, phytochemistry.

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Received On 15 June, 2022

Revised On 5 August, 2022

Accepted On 20 August, 2022

Published On 1 November, 2022

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Chitra Devi K, Subhashini G, Sundar S. K. and Rajan S , Pharmacognostic and Phytochemical Features of Cleome gynandra Whole Plant and its Powder.(2022).Int. J. Life Sci. Pharma Res.12(6), L36-46 http://dx.doi.org/10.22376/ijpbs/lpr.2022.12.6.L36-46

1. INTRODUCTION

Standardization of crude drug is a prominent research in the field of pharmacology. Pharmacology is concerned with the preparation of medicine for the treatment of microbial as well as metabolic disorders. Indian system of medicine uses medicinal plants as a major source of drug. Crude drugs from nature is the backbone of the Indian System of medicine like Siddha, Ayurveda etc.,[1]. In Indian Culture people uses crude preparation as a medicine. One medicinal plant had different name in different places within a country. In most of the cases medicinal plants are consumed without knowing its identity. Now a day medicinal crude drugs are available in the local market with local vernacular names. Sometime unknown use of these drugs may create severe side effects or even death. Improperly used medicinal drugs are ineffective in treatment. Sometimes the crude drugs are in an adulterated, adulteration in crude drug may leads to unwanted effects as well as no drug efficacy[2]. To understand the role of medicinal plants and its identity, the present study is aimed at the assessing the quality of raw drug from the wild and standardize its quality parameters. The objective of this study is to assesses quality of medicinal plant drugs via pharmacognostic, physiochemical, histochemical, fluorescent and phytochemical parameters. In this study, the whole plant of *C. gynandra* was collected from Mannargudi and assessed for its standard pharmacognostic features. This plant is commonly called as Nalvezhalai, and is a member of Cleomaceae family. It is an annual herb, widely spread in many tropical and sub-tropical parts of the world. It is an erect glandular-pubescent annual herb, popularly used in the Ayurveda, Siddha, Folk and Tibetian systems of medicine[3,4]. In India, this plant grows best during august – November months. This plant is indigenous to the tropical and pan tropical regions and plays an important role in agricultural and nutritional system of these regions[5]. Both leaves and flowers of this plant are edible. The leaves have a strong bitter, sometimes peppery flavor similar to mustard greens. This plant's morphology is similar to *Cleome viscosa*, and in Tamil it is called Naikaduku. When the plant is in growing condition, one should differentiate *Cleome* species based on the leaf arrangements, flower and seed pods. After harvesting, it is not possible to differentiate the plant based on the morphological features[6,7]. In this context, pharmacognostic studies are mandatory because once the plant is dried and made into powder form, it loses its morphological identity and is easily prone to adulteration. Such studies would assist in the authentication of medically potential plants and would ensure reproducible quality of herbal products leading to safety and efficacy of natural products. Hence in this study crude drug from *C. gynandra* was subjected for pharmacognostic standardization by making use of the following materials and methods.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The plant of *C. gynandra* was collected from the road sides of SH 66 from Kattur to neivasal road Tamil Nadu. The plant was identified by local people of that village and authenticated by Professor Dr. John Britto, Taxonomist, Department of Botany, St. Joseph's College, Tiruchirapalli, India. A voucher specimen of the plant material has been submitted to herbarium deposit with reference No. 148/Herb/A/20-21. After authentication, the whole plant was shade dried and then milled into coarse powder by a mechanical grinder.

2.2 Powdered Microscopy

Powdered plant material was assessed microscopically for the presence of specific structures. Small quantity of different plant powder was placed separately on slides and each slide was mounted 2-3 drops of chloral hydrate and each slide was covered with cover slip and then examined under microscope. Different cell components were noted and photography was done by using digital camera[8].

2.3 Organoleptic Evaluation

Organoleptic evaluation refers to evaluation of the formulation by colour, odor, taste, texture, etc. The organoleptic characters of the samples were evaluated based on the textual methods[9].

2.4 Physicochemical Parameters

The determination of various physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value, swelling index, foaming index, foreign matters were analyzed by the methods given in the ayurvedic Pharmacopoeia of India[10].

2.5 Fluorescence Analysis

Powder of *C. gynandra* were subjected to analyze fluorescence features under ultraviolet light and daylight after giving treatment for 48 hours with various chemical and organic solvents like ethanol, 50% sulphuric acid, 10% sodium hydroxide and dilute hydrochloric acid [11-13].

2.6 Microbial Limit Assay

Dissolved 1gm of powdered plant material in 10mL of distilled water. It was serially diluted using a phosphate buffer as diluent. The sample was inoculated in Nutrient agar by pour plate, Rose Bengal agar and SS agar by spread plate techniques for Bacteria, Fungi and Salmonella respectively. For bacteria, the plates were incubated at 37°C for 48 hrs and for fungi; the plates were incubated 25°C for 96 hrs[8].

2.7 Qualitative Phytochemical Screening

Preliminary phytochemical characterization was carried out by using standard procedure[14- 16].

2.8 Quantitative Analysis of Phytochemicals

2.8.1 Determination of Total Phenols by Spectrophotometric Method

Total phenols were estimated by the method of Edeoga *et al*.,[17]. Plant powder (2g) was boiled with 50ml of ether for the extraction of the phenolic component for 15min. 5ml of the extract was pipetted out into a 50ml flask, then 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30min for colour development. This was measured at 505nm.

2.8.2 Determination of Tannin

Tannin was determined by Van-Burden and Robinson method[18]. 500mg of the sample was weighed into a 50ml

plastic bottle. 50ml of distilled water was added and shaken for 1hr in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filtrate was pipetted out into a test tube and mixed with 2ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120nm within 10min

2.8.3 Determination of Saponin

Saponin is determined by the method of Obdoni and Ochuko[19]. Plant powder was grounded and 20g of each were kept in a conical flask and 100cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4hr with continuous stirring at about 55° C. The mixture was filtered and the residue re-extracted with another 200ml of 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90° C. The concentrate was transferred into a 250ml separating funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered, while the ether layer was discarded. The purification process was repeated. 60ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant.

2.8.4 Determination of Flavonoid

Flavonoid determined by the method of Boham and Kocipai-Abyazan[20]. 10g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

2.8.5 Histochemical Tests [21,22]

A small quantity of dried and finely powdered bean sample was placed on a grease free microscopic slide and treated with specific chemicals and reagents and waited for 1-2 minutes. A positive test for histochemical was indicated by the appearance of the appropriate colour change after application of the reagent. Light microscope was used to observe and record any colour changes. The powder sample was treated with dilute ammonia and H_2SO_4 gave yellow colour indicating flavonoids. Plant powder treated with dragendorff reagent gave brown colour indicating alkaloids. Plant powder treated with ferric chloride gave Dark blue to black which indicates the presence of tannin. Plant powder treated with 5 drops of acetic anhydride and 5 drops of H_2SO_4 to give Violet to Blue (or) Green colour which indicates the presence of steroids. Plant powder treated with Toludine blue to give Blue green/Red colour indicates the presence of polyphenol. Plant powder treated with Dinitrophenylhydrazine (few drops) to give Orange colour indicates the presence of Terpenoids. Plant powder treated with H_2SO_4 (few drops) to give Yellow colour indicates the presence of Saponin. Plant powder treated with Acidic acid, few drops of ferric chloride and H_2SO_4 to give Brown colour indicates the presence of Glycoside.

2.8.6 Gc-Ms Analysis

GC MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter is 0.32mm, column length is 30m, column thickness 0.50µm), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1.73 ml /min and an injection volume of 0.5 µl was employed (split ratio of 10:1) injector temperature 270 °C; ion-source temperature 200 °C. The oven temperature was programmed from 40 °C (isothermal for 2 min), with an increase of 8 °C/min, to 150°C, then 8°C/min to 250°C, ending with a 20min isothermal at 280°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 51.25min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0[23-25].

2.8.7 Identification of Components

The mass spectrum was interpreted with the aid of the database of National Institute Standard and Technology, WILEY8 and FAME which possess 65,000 plus patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST08s, WILEY8 and FAME library. The name, molecular weight and structure of the components of the test materials were ascertained[26].

3. RESULTS

Quality control of any materials used in the health industry needs to be validated with standard Pharmacognostic studies like organoleptic evaluation, physicochemical assessment, powder analysis and phytochemical analysis. All these methods are useful for checking drug identity and quality of raw materials used in this study. Plant materials were collected from road sides of SH 66 and processed at the Microbiology laboratory of M. R. Government Arts College, Mannargudi. Plant materials were authenticated by botanists and processed. *C. gynandra* is a dense herb available during the August to November period of every year.

3.1 Taxonomy of C. gynandra

This plant belongs to the Kingdom Plantae, Phylum spermatophyta, Division Magnoliophyta, Class Magnoliopsida, Order Brassicales and Family Cleomaceae [27, 28]. Out of 200 species in *Cleome*, 15 are found in India[29, 30]. Genus *Cleome* L and the species *C. gynandra* (L.) Briq.

3.2 Morphology of Cleome gynandra

Cleome species are annual or perennial herbs. Stems are simple or sparsely branched, glabrous or glandular pubescent, foetid or sometimes with scattered prickly appendages. Leaves are 5 foliate pinnately compound; leaf stalk is 20–50 mm long with glandular hairs. Inflorescence racemes, solitary to many flowered, terminal or axillary. Flowers bisexual, zygomorphic or rarely actinomorphic, pedicellate, bracts membranous or leaflike, caducous or persistent, sepals 4, equal, valvate, free or slightly fused at the base, subtending nectary glands at the base, pubescent, petals 4, equal or unequal, usually clawed at base, longer than the sepals. Stamens 4-6, filament inserted on a discoid, declinate, glabrous, anthers linear oblong. Ovary superior, bicarpellary, sessile or on short gynophore, unilocular, ovules many on parietal 2 placentae, style short or

absent, stigma capitate. Fruit capsule, dehiscent, linear oblong, with persistent seed-bearing replum. Seeds 4-40, orbicular or reniform, cleft fused between 2 ends, surface smooth, reticulate or warty, embryo straight (Plate I – Fig. 1-3).

Plate I

***Cleome gynandra* in habit and its powder**

Fig 1-3: *C. gynandra* is an erect yellowish green herb, stem is glandular in nature, leaves are 3-5 foliate, leaflet elliptic oblong and entire, Flowers are white in colour with Corymbose-racemes type (Plate I – Fig 1-3).

Powder microscopy revealed the presence of a xylem element, which showed dense spiral lining lateral wall thickenings. There are wide rectangular parenchyma cells which form one below the other forming vertical strands. The cell wall is fairly thick and inside cells are seen as dark particles. There are other types of parenchymal cells which are wider with thick walls and dense cytoplasm. There are fibres with varying thickness and length. This is another vessel element which has a long conical and thick tail at one end. The other end is blunt. There are wide circular perforations. The cell has thick cell wall and there are numerous circular bordered pits. In the powder, bundles of fibres and parenchymal cells were seen. They are occurring in vertical compact rows. The fibres are narrow thick walled with narrow lumen. The parenchyma cells vertically rectangular compactly arranged one below another. Some of the parenchymal cells exhibit prominent nucleus. Many parenchyma cells are seen which are long, narrow and wide. The narrow parenchyma cells are separated and two celled. It has prominent nuclei. Some parenchymal cells are wide, long and septate. There are also fibres which are narrow thick walled and lignified (Plate II – Fig. 4-7). Size of the various elements found in *C.gynandra* were in Table 1.

Table 1: Microscopic characters of Powder of *C. gynandra* whole plant powder

Xylem element	600µm long and 80 µm thick.
The strand is, the	140 µm long
individual cells	40 µm long and 20 µm wide.
Parenchyma cells	70 µm long and 40 µm wide.
The vessel element	110 µm long and 50 µm thick

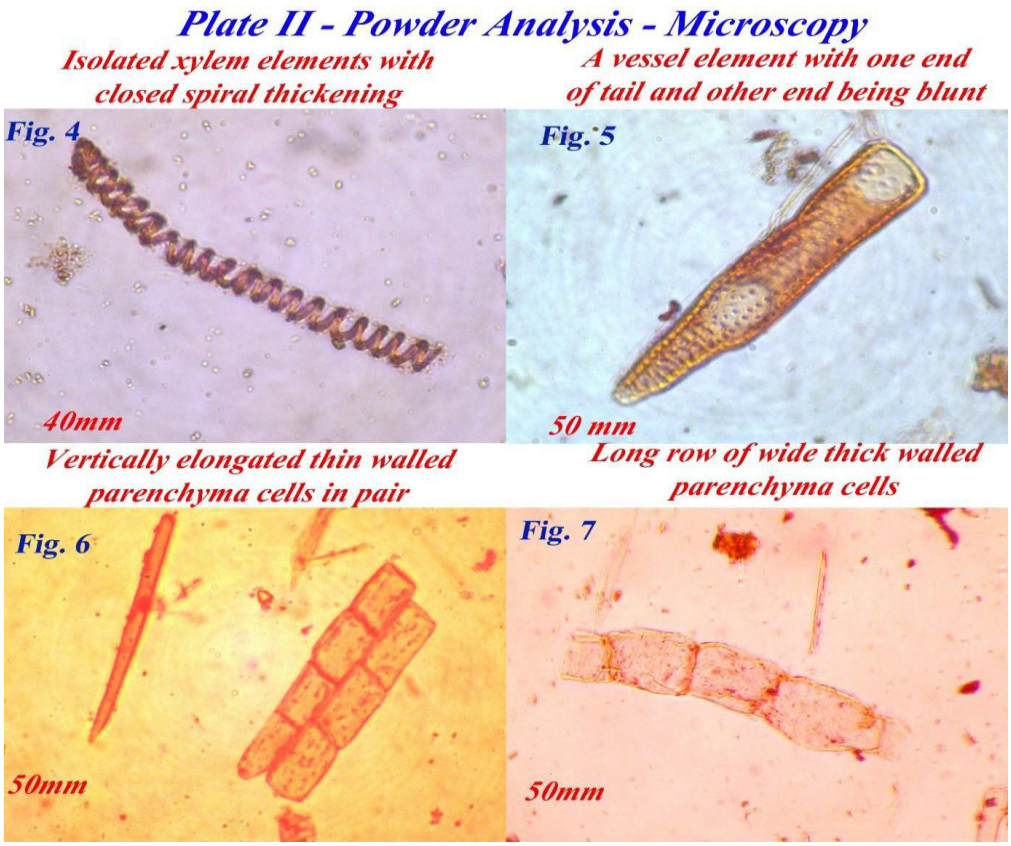

Fig. 4 -7 - Presence of a xylem element, which showed dense spiral lining lateral wall thickenings. There are wide rectangular parenchyma cells which form one below the other forming vertical strands (Plate II – Fig. 4-7)

3.3 Ayurvedhic properties of C. gynandra [31]

Medicine made from this plant is used for the treatment of various diseases, which is illustrated in ayurvedic texts. In Sanskrit this plant is called Ajagandha.
Vahnikrut – Improve digestion strength
Hrudya – acts as a cardiac tonic.
Ruchya – Improves taste.
Druk – Good for eyes
Shukrhara – Aphrodisiac.
Kaphavatahara- Balances Kapha and vada doses
Grahi – Absorbent, useful in diarrhoea.
Kaphapaha – useful in productive cough, asthma
Vatahara – Treat paralysis, constipation
Pittala – Controls Pitta dosha
Gulma – tumours of the abdomen

Table 2- Organoleptic characters of mixed powder preparation of C. gynandra

S. No	Character	Observation/ Result
1	Colour	Yellowish Green
2	Odour	Characteristic
3	Taste	Bitter
4	Texture	Rough

Processed whole plant of *C. gynandra* was assessed for its organoleptic characters, it revealed that the powder was greenish yellow in colour, characteristic odour, bitter taste and rough texture (Table 2).

Table 3 - Physicochemical constant and extractives of mixed powder preparation of *C. gynandra*

S. No	Parameter	Results
1	Foreign matter	Nil
2	Total ash	04.9%
3	Acid insoluble ash	01.2%
4	Water soluble ash	02.3%

5	Water extractive	23.7%
6	Alcohol extractive	18.3%
7	Chloroform extractive	04.2%
8	Ethyl acetate extractive	02.1%
9	Hexane extractive	00.8%

When powder was analysed for its physicochemical parameters, it showed 4.9% total ash content followed by 2.3% w/w water soluble ash and 1.7% acid insoluble ash. This is within the limits of ayurvedic pharmacopeia of India (Table 3). Extractive phytocompounds play a vital role in biological potentials. Mixed powder preparation showed 23.7%w/w water extractives followed by 18.3%w/w ethanol extractive, 4.2% chloroform extractive, 2.1% ethyl acetate extractive and 0.8%w/w hexane extractives. Higher water extractive indicated that the presence of polar phytochemicals in the powder. This was done successively with increased polar solvents. This is also in line with ayurvedic pharmacopoeia of India.

Table 4- Fluorescence analysis of *C. gynandra*

S. No	Test	Visible Light	Short UV (254 nm)	Long UV (365 nm)
1	Plant powder	Green	Green	Black
2	Plant powder +Water	Green	Light yellowish green	Black
3	Plant powder+Hexane	Green	Green	Black
4	Plant powder+Chloroform	Green	Dark green	Black
5	Plant powder+Methanol	Green	Black green	Black
6	Plant powder +Acetone	Black green	Black	Black
7	Plant powder+1N NaOH	Yellowish green	Yellowish green	Black
8	Plant powder+1N HCL	Brownish black	Brown	Black
9	Plant powder+H2SO4+Water	Black	Black	Black
10	Plant powder+HNO3+Water	Black	Brown	Violet brown

The chromogenic nature of phytochemicals was assessed by fluorescence analysis which indirectly revealed the presence of phytochemicals present in the plant powder. The powder of *C. gynandra* showed mostly black colouration under long wavelength of UV (365nm). Powder preparations observed under visible light showed characteristic green, dark green, yellowish green, blackish brown, yellowish brown and brown colouration (Table 4).

Table 5 - Nature of microbial availability in mixed powder preparation of *Cleome gynandra*

S. No	Test organism	Microbial count
1	Total aerobic Bacteria	67 x 10^2 CFU/g
2	Total Fungal count	14 CFU/g
3	Total Enteric Bacteria	Nil
4	Total *E. coli*	Nil
5	*Salmonella*	Nil
6	*Shigella*	Nil

Microorganisms play a vital role in maintaining quality of raw as well as processed drug materials. Microbial quality needs to be assessed for the better utility as human medicine. Standard methods assessed low quantity of microbial load. Powder showed only 67x10^2 CFU/g of total count of aerobic bacteria and 14 CFU/g of total fungus. There are no enteric bacteria like *Escherichia coli, Salmonella* and *Shigella* (Table 5). This is also within the limits of ayurvedic pharmacopoeia of India.

Table 6 - Phytochemicals qualitative analysis of *Cleome gynandra*

S. No	Phytochemicals	Result
1	Tannin	++
2	Saponin	+
3	Flavonoids	++
4	Steroids	++
5	Terpenoids	+
6	Triterpenoids	+
7	Alkaloids	+
8	Anthroquinone	+
9	Polyphenol	++
10	Glycoside	++
11	Coumarins	+
12	Emodins	-
13	Anthocyanins	+

(-) Absent, (+) Present and (++) high concentration

Table 7 - Quantitative analysis of *Cleome gynandra*

S. No	Phytochemicals	Results (mg/gm)
1	Polyphenol	162.57 ± 7.87
2	Flavonoids	94.63 ± 4.98
3	Tannin	35.46 ± 2.08
4	Saponin	50.15 ± 3.41

Values expressed as Mean ± SD for triplicates

The results of preliminary qualitative phytochemical screening of whole plant powder preparation of *C. gynandra* revealed the presence of multiple polar and non-polar chemical constituents (Table 6). Saponins, Tannins, Flavonoids, steroids, terpenoids, triterpenoids, alkaloids, anthraquinones, polyphenols, glycosides, coumarins and anthocyanins in the powder. Similar compounds were found in aqueous extract also but alkaloids and anthocyanins are absent in *C.gynandra* whole plant. Quantitative phytochemical nature was expressed that the extract showed higher quantities of poly phenols (Table 7), followed by flavonoids, tannins and saponins.

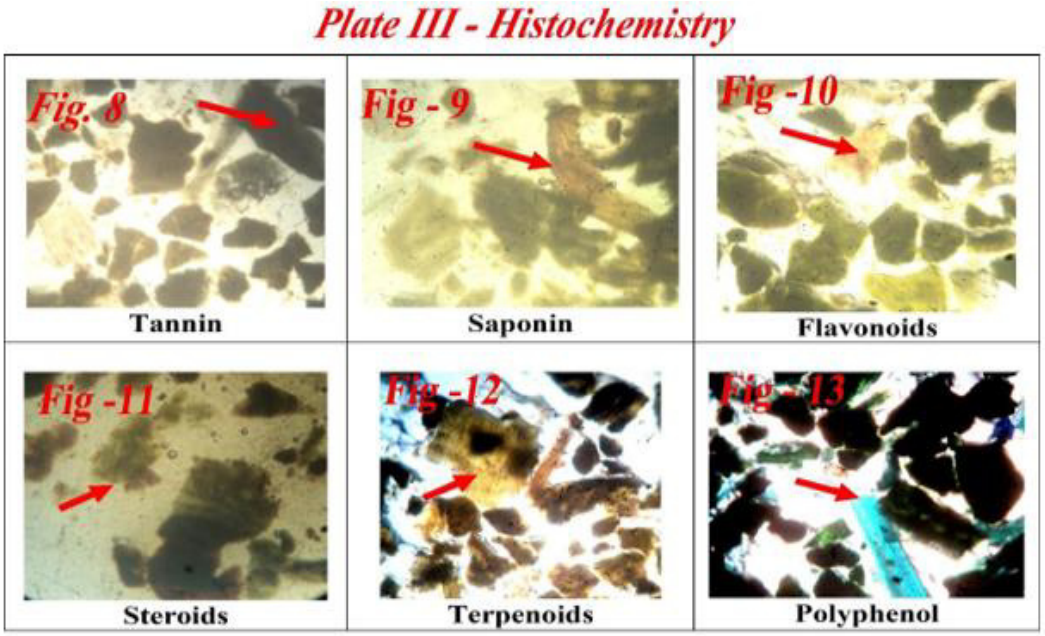

Fig. 8-13 – Histochemistry - Flavonoids -yellow, alkaloids -brown colouration; Tannins - blue colouration (Plate III – Fig. 8-13).

Table 8- Histochemical analysis of *Cleome gynandra*

S. No	Phytochemicals	Histochemical Results	
		Colour observation	Results
1	Tannin	Black	++
2	Saponin	Yellowish green	++
3	Flavonoids	Yellow	++
4	Steroids	Blue to light green	+
5	Terpenoids	Orange	+
6	Polyphenol	Green/Blue	++

(-) Absent, (+) Present and (++) high concentration

This is also confirmed in histochemical analysis (Table 8). The histochemical features express the following, Flavonoids are detected on the basis of yellow colouration with diluted ammonia and H_2SO_4. Presence of alkaloids are indicated by the presence of brown colouration by the addition of Dragendorff's reagent. Dark blue colour formation with ferric chloride indicates the presence of tannin. Plant powder treated with 5 drops of acetic anhydride and 5 drops of H_2SO_4 to give Blue to light green colour indicates the presence of steroids. Toludine blue treatment with plant powder gives bluish green colour indicating the presence of polyphenol. Orange colouration with Dinitrophenylhydrazine (few drops) showed the presence of Terpenoids. Plant powder treated with few drops of sulphuric acid provides the presence of saponins with the formation of yellow colour (Plate III – Fig 8-13). Twenty compounds were detected in ethanol mixed crude preparation of *C. gynandra* via GC-MS peak analysis (Plate IV -Fig. 14 and Table 9, 10). The prevailing compounds are 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-,9,12-Octadecadienoic acid (Z, Z), 9,12,15-Octadecatrienoic acid and methyl ester, (Z, Z, Z). The presence of various bioactive compounds justifies the use of the plant for various ailments by traditional practitioners. Table 10 illustrates the bio efficiency of individual compounds with reference to Dr. Dukes database[26].

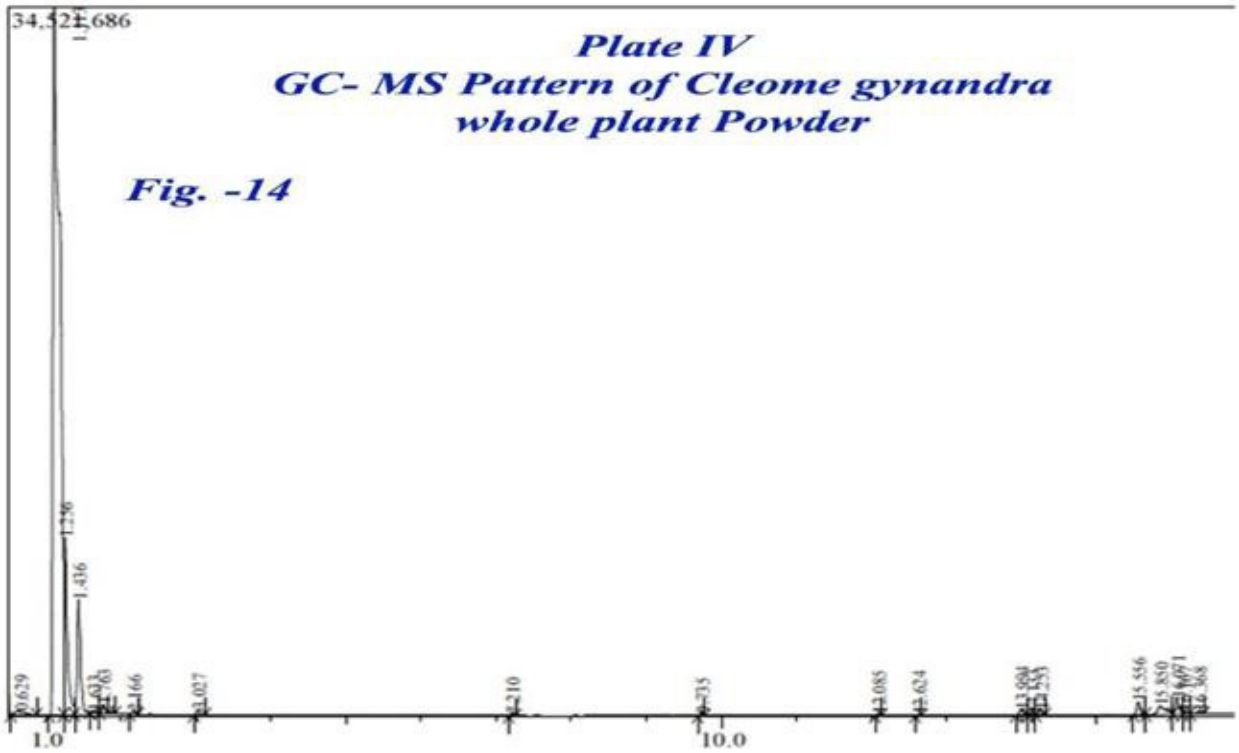

Fig. 14 – GC MS pattern - The prevailing compounds are 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-,9,12-Octadecadienoic acid (Z, Z), 9,12,15-Octadecatrienoic acid and methyl ester, (Z, Z, Z) (Plate IV – Fig. 14)

Table 9: Identification of active compounds in *Cleome gynandra* - GCMS

Peak #	R. Time	Area%	Height%	M. weight (g/mol)	Molecular formula	Name
1	0.629	0.67	0.34	170	$C_{10}H_{16}D_2O_2$	Methyl-7,8-dideutero-7-nonenoate
2	1.113	78.24	65.29	92	$C_2H_4O_2S$	Acetic acid, mercapto-
3	1.256	8.72	16.31	46	C_2H_6O	Ethanol
4	1.436	7.17	10.57	118	$C_6H_{14}O_2$	Ethane, 1,1-diethoxy
5	1.633	0.10	0.13	142	$C_9H_{18}O$	5-ethyl-2-heptanone
6	1.763	0.46	0.80	144	$C_8H_{16}O_2$	1,1-Diethoxy-2-butene
7	2.166	0.15	0.30	99	$C_4H_5NO_2$	Pyrrolidine-2,4-dione
8	3.027	0.27	0.51	130	$C_8H_{18}O$	1-Hexanol, 2-ethyl
9	7.210	0.06	0.15	196	$C_{14}H_{28}$	3-Tetradecene
10	9.735	0.08	0.19	224	$C_{16}H_{32}$	3-Hexadecene
11	12.085	0.06	0.15	587	$C_{36}H_{75}O_3P$	Phosphonic acid, dioctadecyl ester
12	12.624	0.05	0.11	184	$C_{12}H_{24}O$	Cyclododecanol
13	13.994	0.40	0.51	282	$C_{18}H_{34}O_2$	9-Octadecenoic acid
14	14.133	0.10	0.14	232	$C_{12}H_{12}N_2O_3$	2-([(1-Cyano-1-ethylethyl)amino] carbonyl)benzoic acid
15	14.253	0.26	0.52	270	$C_{17}H_{34}O_2$	Pentadecanoic acid, ethyl ester
16	15.556	0.63	1.19	296	$C_{20}H_{40}O$	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-
17	15.850	1.06	0.77	280	$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid
18	16.071	1.15	1.42	430	$C_{25}H_{34}O_4S$	2,3,6,7-tetramethyl-10-(4-methylphenylsulfonyloxy)-1,4,4.alpha.,5,8,8a.beta.,9.beta.,9a.beta.,10.alpha.,10a.alpha.-decahydroanthracen-9-ol
19	16.167	0.17	0.32	292	$C_{19}H_{32}O_2$	9,12,15-Octadecatrienoic acid, methyl ester
20	16.368	0.20	0.28	270	$C_{17}H_{34}O_2$	Pentadecanoic acid, ethyl ester

Table.10: Biological activity of compounds identified in *Cleome gynandra* powder extract using GC-MS

R. Time	Name of the compounds	Biological activity**
13.997	9-Octadecenoic acid	Cancer preventive, Anti-inflammatory, Hypocholesterolemic, Antiarthritis, Antihypertensive, Increase HDL and decrease LDL Cholesterol.
15.556	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl	Precursor for the manufacture of synthetic forms of vitamin E and vitamin K1. used in the fragrance industry and used in cosmetics, shampoos, toilet soaps, household cleaners, and detergents.
15.850	9,12-Octadecadienoic acid	Antiinflammatory, Nematicide, Insectifuge,

		Hypocholesterolemic, Cancer preventive, Hepatoprotective, Antihistaminic, Antiacne, Antiarthritic, Antieczemic, 5-Alpha reductase inhibitor, Antiandrogenic, Anticoronary.
16.167	9,12,15-Octadecatrienoic acid, methyl ester	Hypocholesterolemic, Nematicide Antiarthritic, Hepatoprotective, Anti androgenic, Nematicide 5-Alpha reductase inhibitor, Antihistaminic Anticoronary, Insectifuge, Antieczemic, Anticancer.

***Source: Dr. Duke's phytochemical and ethnobotanical databases [Online database[26]].**

4. DISCUSSIONS

C. gynandra are abundantly found in Africa and Asia and showed slight morphological variation[32], which could be due to nutrient content of soil, climate and other ecological parameters [33, 34] The characteristics of the different plant parts of C. gynandra were clearly described by scientists from different parts of the country- [36], which confirms the identity of the plant materials. Mature plant *C. gynandra* L. is an herbaceous, erect, and annual plant that grows to a height ranging from 0.5 m to 1.5 m at maturity, depending on the growing environment. The stem of *C. gynandra* is sticky with glandular hairs, marked with longitudinal parallel lines. The stem pigmentation varies from green to pink and purple[37-40]. *C. gynandra* belongs to the family Cleomaceae, which is confirmed after different taxonomic arguments[41,42]. It is found in wastelands and road sides[43], similarly plants used in this study were also collected from the road sides of Thiruvarur district SH66. Morphologically, this plant is an erect herb, with compound five foliate leaves and it showed 20-50mm leaf stock with glandular hairs, flowers are white in colour (Platel). Trichome and calcium oxalate cuboid crystals confirmed this plant belongs to the family Cleomaceae and the genus Cleome species[6]. Assessment of the ash value is useful in detecting adulterations. Low ash content detected in powder preparation showed the availability of lower quantity or absence of inorganic impurities like soil[44]. Minimal ash value indicates that the raw drug is free from foreign matter. The extractive values with water and ethanol confirms the holistic usage of medicinal plants, it also indirectly illustrated the presence of specific polar phyto constituents of the plant material[42,45]. Flavonoids, protein, carbohydrates were the reason for higher water extractive value. Lower hexane extractable value indicated minimal availability of straight chain fatty acid. Chromophore nature of the plant materials were expressed via fluorescence analysis test, which indicated the presence of multipolar phytoconstituents in the extracts. Behavior of drug materials under UV radiation and visible light exhibited different colour depending on the various chromophores present in the material. The same extract may appear different at different wavelength of light[12-14]. *C. gynandra* extracts showed higher concentrations of phenolic compounds and flavonoids which was also confirmed by Farjana et al [46] and Katsube et al., [47] indicated that these compounds possess antidiabetic and antioxidant activity. Polyphenols like phenolic acids, flavonoids and tannins were found in higher concentrations which are responsible for numerous pharmacological activities[48]. Presence of phenolic compounds and tannins in this plant part supports chemically the antidiarrheal activity and antimicrobial activity, which was in agreement with various earlier reports[49,50]. Phenolic compounds are used in the treatment of burns as they precipitate the proteins of exposed tissue to form a protective covering[51]. They are also used as healing agents in inflammation, leucorrhoea, gonorrhoea, burns, piles and as antidote[52, 53]. Microbial limit assay indicated quality of raw or crude drug powder, number of microbial cells are directly proportional to the quality of the plant materials[54-56]. GC-MS data revealed most of the compounds were fatty acids with phenolic compounds and flavonoids. This study is in line with the quality parameters prescribed in Ayurvedic Pharmacopoeia of India and also standards set by other international agencies. This work provides qualitative and quantitative standards for the identification of plant powder.

5. CONCLUSION

The present study confirmed the identity of plants under study using morphological observation. This study also indicated proper pharmacognostic and crude powder analysis, which could be considered as a standard for the crude drug from nature. Phytochemical identity also confirmed via histochemical and phytochemical study. Results of this study could be considered as a quality standard for whole plant powder of *C. gynandra*.

6. AUTHOR CONTRIBUTION STATEMENT

Mrs. Subhashini and Mrs Chitradevi hypothesized the study and performed all experiments, Dr. S. Rajan analyzed all data and discussed all study matters and Dr. S. K. Sundar designed all the methodology described in the study. All the authors read this article and approved it.

7. ACKNOWLEDGEMENT

The authors are grateful to Principal, M. R. Government Arts College, Mannargudi. Mr. Chelladurai from Government Siddha college, Tirunelveli is gratefully acknowledged for helping in initial identification of plant materials.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

9. REFERENCES

1. Mishra SS, Moharana SK, Dash MR. Review on *Cleome gynandra*. Int J Res Pharm Chem. 2011;1:681-9.
2. Khandelwal KR. Practical pharmacognosy. Nirali Prakashan, Pune. 16th ed; 2007. p. 149-56.
3. Moyo M, Amoo SO, Aremu AO, Gruz J, Šubrtová M, Jarošová M et al. Determination of mineral constituents, phytochemicals and antioxidant qualities of *Cleome gynandra*, compared to Brassica oleracea and

Beta vulgaris. Front Chem. 2017;5:128. doi: 10.3389/fchem.2017.00128, PMID 29354633.
4. Sivakumar D, Chen L, Sultanbawa Y. A comprehensive review on beneficial dietary phytochemicals in common traditional southern African leafy vegetables. Food Sci Nutr. 2018;6(4):714-27. doi: 10.1002/fsn3.643, PMID 29983933.
5. Sandhya G, Shanmugavadivu M, Vidhya J. Preliminary phytochemical screening, antibacterial and thrombolytic activity of *Cleome gynandra* aqueous extract. Int J Adv Resin Biol Sci. 2018;5(3):30-6.
6. Uday D, Ujjwal L. Pharmacognostic and phytochemical evaluation of Cleome viscosa L. (Cleomaceae). Int J Creat Res Thoughts. 2021;9(9):491-9.
7. Yoganarasimman SN. Medicinal plants, Regional research institute (AY), Bangalore. 2000;2:343, 452.
8. Anonymous. Indian pharmacopoeia. Gov India Minist Health Fam Welf Controller Publ Civ Lines Delhi–110054. 1996;I & II.
9. Siddiqui A, Hakim MA. Format for the pharmacopoeial analytical standards of compound formulation, workshop on standardization of Una ni drugs, (appendix), 24-25 January. New Delhi: central council for research in Una ni Medicine (CCRUM); 1995.
10. Wallis TE. Textbook of pharmacognosy. 5th ed, CBS Publishers and Distributors. New Delhi, India; 2005.
11. Kokate CK. Practical pharmacognosy. 1st ed. New Delhi: Vallabh Prakashan; 1986. p. 111.
12. Chase CR, Pratt RS. Fluorescence of Powdered Vegetable drugs with particular reference to Development of a System of Identification. JAmerican. Pharmacol. Association. 1949;38:32.
13. Brindha P, Sasikala P, Purushothaman KK. Pharmacognostic studies on merugan kizhangu. Bull ETH Bot Res. 1981;3:84-96.
14. Sofowara A. Medicinal plants and Traditional medicine in Africa. Ibadan, Nigeria: Spectrum Books Ltd; 1993. p. 289.
15. Trease GE, Evans WC. Pharmacognsy. Brailliar Tiridel can. 11th ed. Macmillian publishers; 1989.
16. Harborne JB. Phytochemical Methods.A Guide to Modern Technique of Plant analysis. London: Chapman & Hall; 1984. p. 78-210.
17. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol. 2005;4(7):685-8. doi: 10.5897/AJB2005.000-3127.
18. Van-Burden TP, Robinson WC. Formation of complexes between protein and tannin acid. J Agric Food Chem. 1981;1:77-82.
19. Obdoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria. Glob J Pure Appl Sci. 2001;8:203-8.
20. Boham BA, Kocipai-Abyazan R. Flavonoids and condensed tannins from leaves of Vaccinium vaticulatum and V. calycinium. Pac Sci. 1974;48:458-63.
21. John Peter Paul J. Histochemistry and fluorescence analysis of Turbinaria ornata (Turner) J. Ag. –An important brown seaweed (Phaeophyceae). Ind J Plant Sci. 2014;3(1):40-4.
22. Gersbach P, Wyllie VG, Sarafis V. A new histochemical method for localization of the site of monoterpene phenol accumulation in plant secretory structures. Annals of Botany. 2001;88(4):521-5. doi: 10.1006/anbo.2001.1480.
23. Srinivasan K, Sivasubramanian S, Kumaravel S. Phytochemical profiling and GC-MS study of Adhatoda vasica leaves. Int J Pharm Biol Sci. 2013;5(1):714-20.
24. Ashalatha KS, Raveesha HR. Phytochemical screening, GC-MS & HPLC analysis of Decalepis Arayalpathra (Joseph & Chandras) an endangered medicinal plant. Int J Pharma Bio Sci. 2020;10(4):L64-72. doi: 10.22376/ijpbs/lpr.2020.10.4.L64-72.
25. Mohan B, Nayak JB, Kumar RS, Shiva Kumari LP, Mohan Ch, Rajani B. Phytochemical screening, GC-MS analysis of Decalepis hamiltonii Wight & Arn. an endangered medicinal plant. J Pharmacogn Phytochem. 2016;5(5):10-6.
26. Dr.duke's Phytochemical and Ethno botanical Databases, Phytochemical and Ethnobotanical Databases; 2013. Available from: http://www.ars-gov/cgi-bin/duke/.
27. Conrad LS, Stacy C, Mikhail D, Carol LH, Sivakumar K, Rogneda K et al. NCBI Taxonomy: a comprehensive update on curation, resources and tools. Database (Oxf). 2020;1:baaa062. doi: 10.1093/database/baaa062.
28. Raju AJS, Rani DS. Reproductive ecology of *Cleome gynandra* and Cleome viscosa (Capparaceae). Phytol Balcanica. 2016;22(1):15-28.
29. Iltis HH, Cochrane TS. Studies in the Cleomaceae V: A new genus and ten new combinations for the Flora of North America. Novon. 2007;17(4):447-51. doi: 10.3417/1055-3177(2007)17[447:SITCVA]2.0.CO;2: A J Botanical Nomenclature 17:447-451.
30. Aparadh VT, Karadge BA, Mahamuni RJ. Taxonomy and physiological studies in spider flower (Cleome species): A Critical review. Plant Sci Feed. 2012;2:25-46.
31. Iltis HH, Hall JC, Cochrane TS, Sytsma KJ. Studies in the Cleomaceae I. On the separate recognition of Capparaceae, Cleomaceae, and Brassicaceae I. Ann Mo Bot Gard. 2011;98(1):28-36. doi: 10.3417/2007017.
32. Maundu P. Achigan-Dako E and Morimoto Y. Biodiversity of African vegetables. In: Shackleton CM, Pasquini MW, Drescher AW, editors African indigenous vegetables in urban agriculture. London, UK: Earthscan Publications; 2009. p. 65-104.
33. Tibugari H, Paradza C, Rukuni D. Germination response of cat's whiskers (*Cleome gynandra* L.) seeds to heat shock, potassium nitrate and puncturing. J Agric Technol. 2012;8:2309-17.
34. Mnzava NA, Chigumira FN. *Cleome gynandra* L. In. In: Grubben GJH, Denton OA, editors Plant resources of tropical Africa 2. Vegetables. Wageningen: PROTA Foundation, -Netherlands/ Backhuys Publishers. Leiden, Netherlands: CTA. Wageningen, Netherlands; 2004. p. 191-4.
35. Rensburg WSJ, van Averbeke W, Slabbert R, Faber M, van Jaarsveld P, van Heerden I et al. African leafy vegetables in South Africa. Water South Africa. 2007;33:317-26.
36. Edeoga HO, Omosun G, Osuagwu GGE, Mbaebie BO, Madu BA. Micromorphological characters of the vegetative and floral organs of some Cleome species from Nigeria. Am-Eura J Scienti Res. 2009;4:124-7.
37. Onyango CM, Kunyanga CN, Ontita EG, Narla RD, Kimenju JW. Current status on production and utilisation of spider plant (*Cleome gynandra* L.) an underutilized leafy vegetable in Kenya. Genet Resour Crop Evol. 2013;60(7):2183-9. doi: 10.1007/s10722-013-0036-7.

38. Wasonga DO, Ambuko JL, Cheminingwa GN, Odeny DA, Crampton BG. Morphological characterization and selection of spider plant (*Cleome gynandra*) accessions from Kenya and South Africa. Asian J Agric Sci. 2015;7(4):36-44. doi: 10.19026/ajas.7.2198.
39. Wenyika P, Gasura E, Kafesu N, Goss M, Matikiti A, Kujeke G. Discriminating phenotypic markers reveal low genetic diversity in spider plant. Afr Crop Sci J. 2015;23(4):305-10. doi: 10.4314/acsj.v23i4.1.
40. Omondi EO, Engels C, Nambafu G, Schreiner M, Neugart S, Abukutsa-Onyango M et al. Nutritional compound analysis and morphological characterization of spider plant (*Cleome gynandra*) - an African indigenous leafy vegetable. Food Res Int. 2017;100(1):284-95. doi: 10.1016/j.foodres.2017.06.050, PMID 28873690.
41. Ahouansinkpo E, Atanasso J, Dansi A, Adjatin A, Azize O, Sanni A. Ethnobotany, Phytochemical Screening and Toxicity Risk of *Cleome gynandra* and Cleome viscosa, two traditional leafy vegetables consumed in Benin. Int J Curr Microbiol Appl Sci. 2016;5(2):813-29. doi: 10.20546/ijcmas.2016.502.093.
42. Oshingi S, Fekadu FD, Emmanuel OO, Traud W, Mary OA. *Cleome gynandra* L. origin, taxonomy and morphology: a review. Afr J Agric Res. 2019;14(32):1568-83. doi: 10.5897/AJAR2019.14064.
43. Pillai LS, Nair BR. Proximate composition, mineral elements and anti-nutritional factors in Cleome viscosa L. and Cleome burmanni W. & A. (Cleomaceae). Int J Pharm Pharmaceu Sci. 2013;5:384-7.
44. Gupta PC, Rao CV. Pharmacognostical studies of Cleome viscosa Lin. Ind J Nat Prod Resour. 2012;3(4):527-34.
45. Lala PK. Practical pharmacognosy, 1. New Delhi: Vallabh Prakashan; 1981. p. 86-95.
46. Yasmin F, Yusoff NA, Hossain A. Phytochemical screening and pharmacological activities of the ethanolic stem extract of *Cleome gynandra*. Pak J Nutr. 2020;19(4):153-9. doi: 10.3923/pjn.2020.153.159.
47. Katsube T, Yamasaki M, Shiwaku K, Ishijima T, Matsumoto I, Abe K et al. Effect of flavonol glycoside in mulberry (Morus alba L.) leaf on glucose metabolism and oxidative stress in liver in diet induced obese mice. J Sci Food Agric. 2010;90(14):2386-92. doi: 10.1002/jsfa.4096, PMID 20648552.
48. Morshed MA, Uddin A, Saifur R, Barua A, Haque A. Evaluation of antimicrobial and cytotoxic properties of Leucas aspera and Spilanthes paniculata. Int J Biol Sci. 2011;1:7-16.
49. Ajila CM, Prasada Rao UJ. Protection against hydrogen peroxide induced oxidative damage in rat erythrocytes by Mangifera indica L. seed extract. Food Chem Toxicol. 2008;46(1):303-9. doi: 10.1016/j.fct.2007.08.024, PMID 17919803.
50. Keher, Chanda. Herbal medicine. Curr Sci. 1946;15:48.
51. Toshihide T, Hadjime N, Megumi A, Shigeko U, Yoshiharu K, Shun'ichi D. Characterization of novel antimicrobial compounds from mango (Mangifera indica L.) kernel seeds. Food Chem. 2000;71(1):61-6. doi: 10.1016/S0308-8146(00)00126-6.
52. Handa SS, Sharma A, Chakraborti KK. Natural products and plants as liver protecting drugs. Fitoterapia. 1986;57:307-45.
53. Meda NTR, Bangou MJ, Bakasso S, Millogo-Rasolodimby J, Nacoulma OG. Antioxidant activity of phenolic and flavonoid fractions of *Cleome gynandra* and *Maerua angolensis* of Burkina Faso. J Appl Pharm Sci. 2013;3(02):036-42.
54. Nyarko HD, Barku VYA, Batama J. Antimicrobial examinations of Cymbopogon Citratus and Adiatum capillus-veneris Used in Ghanaian folkloric medicine. Int J Life Sci Pharm Res. 2012;2(2):115-21.
55. Rao KNV, Sunitha C, David B, Sandhya S, Saikumar P. Pharmacognostic studies on Rumex vesicarius. Asian J Plant sci and Res. 2011;2011; 1(1):102-15.
56. Adhikari PP, Paul SB. Medicinally important plant *Cleome gynandra*: A phytochemical and pharmacological explanation. Asian J Pharmaceu and. Clin Res. 2018;11(1):21-9.