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Original Research Article

Phytochemical Screening and Fluorescence Analysis of *Cleome gynandra* L. Leaves

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Abstract

The aim of this study is to determine the phytochemical constituents of ethanol leaf extract of *Cleome gynandra*. Phytochemistry helps in standardizing the herbal preparations and possibly relates the constituents to their medicinal/pharmacological uses. Dried leaf material was grounded to coarse powder and stored in airtight container followed by the extraction with ethanol. The dry powder of leaf sample was observed under U.V. light to evaluate the fluorescence. Phytochemical screening of leaf crude extracts of *Cleome gynandra* in different solvents like methanol, ethanol, petroleum ether, chloroform and acetone. The quantitative studies revealed that leaves of *Cleome gynandra* possessed alkaloids, carbohydrates, glycosides, saponins, phytosterols, proteins, flavonoids and lignin. The preliminary phytochemical screening tests in individual plant or plant part may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and drug development.

Keywords

Cleome gynandra

Fluorescence analysis

Phytochemicals

Introduction

Traditional medicinal practice has been known for centuries in many parts of the world. Herbal medicines are gaining interest because of their cost effective and eco-friendly attributes. Traditional knowledge regarding of medicinal plants and their use by indigenous cultures are not only useful for conservation of cultural traditions and biodiversity but also for community healthcare and drug development in the present and future. Plant and plant extracts have formed important position in modern medicine, due to their chemical and medicinal contents found in the natural form. The secondary metabolites represent a large reservoir of structural moieties which work together exhibiting a wide range of biological activities .The use of higher plants and their extracts to treat infections is an age-old practice. Medicinal plants have been an integral part of the ethno botanical aspects of the people. The modern medicine has evolved from folk medicine and traditional system only after thorough chemical and pharmaceutical screening. Thus, plants remain the major source of medicinal compounds. It has been estimated that about 20,000 plant species are used for medicinal purposes.

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. According to World Health Organization, medicinal plants would be the best source

to obtain a variety of drugs and more than 80% of the world's population relies on traditional medicines for their primary health care needs. The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. The phytochemical research based on ethno pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants. Leaves are regarded as medicinal for the treatment of various ailments like 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, and convulsions and in certain bilious disorders (Van den Heever and Venter, 2007; The Wealth of India, 1956; Chatterjee A and Pakrashi , 1991; Oliver-Bever, 1983; Kumar and Sadique, 1987; Tabuti et al., 2003; Cano and Volpato, 2004; Hebbar et al., 2004; Kamatenesi-Mugisha and Oryem-Origa, 2007).

In the present study an attempt has been made the laboratory evaluations to assess the analytical and phytochemical screening of *Cleome gynandra* Linn. and possibly relate the constituents to their medicinal/pharmacological uses. Phytochemical studies of the plant are necessary for standardization, which helps in understanding the significance of phytoconstituents in terms of observed activities. Phytochemical screening also helps in standardizing the herbal preparations.

Botanical description of the plant

Cleome Gynandra Linn belongs to the family Capparidaceae. It is a herb indigenous to the tropical and pan tropical regions. *It* is used as a green vegetable and known by many common names including Shona cabbage, African cabbage, spider wisp, spider flower and cat's whiskers. It is an annual wild plant native to Africa but has become widespread in many tropical and sub-tropical parts of the world. It is an erect, branching plant generally between 25 cm and 60 cm tall. Its sparse leaves are each made up of 3-5 oval-shaped leaflets. The flowers are white to rose pink. The seed is a brown 1.5mm diameter sphere. The leaves are edible. The leaves form an important part of diets in Southern Africa, and nutritional analysis has found it to be high in certain nutrients including amino acids, vitamins and minerals. *Cleome gynandra* is closely related to *Arabidopsis thaliana* and therefore offers comparison with this well studied model organism.

Distribution of the plant

Cleome gynandra is a common, widespread herb occurring in southern Africa extending from the Limpopo, the North-West, Gauteng, Mpumalanga, KwaZulu-Natal, Free State, the Northern Cape and Namibia. It is probably a native of Africa and now widely distributed in tropical and subtropical regions throughout the world. The natural habitat of *Cleome gynandra* is waste land and arable land with annual species as well as grasslands. The species have a C4 photosynthetic pathway, an adaptation mechanism that enables it to survive in drier and hot environments. It grows well up to about 1000 MSL in semiarid, sub humid and humid climates, and is adapted to many soil types. *Cleome gynandra* is an abundantly available species and grows as a weed in common barren land and in crop fields throughout India .In all over the world in different countries it is used to treat many diseases in their traditional system and it is also used in various traditional culinary systems for its remarkable nutritional and antioxidant properties. In India alone it is used by the traditional healers for many diseases e.g. .epilepsy, irritable bowel syndrome and in protozoal and worm infections. The high protein and amino acids, minerals content of this plant can make this as a highly economically important that can be grown and cultivated easily.

Cleome gynandra is used as a vegetable where the tender shoots and leaves are boiled and eaten as herb, tasty relish, stew or side dish. The vegetable is a rich source of protein, and the leaves contain over and above the normal recommended adult daily allowance of vitamins A and C and the minerals calcium and iron. Sap from leaves has been used as an analgesic particularly for headache, epileptic fits and ear ache while bruised leaves, which are rubefacent and vesicant, are also used to treat neuralgia, rheumatism and other localized pains (Narendhirakannan et al., 2003 and 2005). Leaves and seeds of *Cleome gynandra* is used for earache, epileptic fits, stomach-ache, constipation and inflammation in most of the countries. Fresh leaves of

Cleome gynandra is used in ayurveda and siddha medicine for a variety of disease conditions. The plant material is used as an anthelmintic drug in ayurveda for ear diseases, pruritus and several other diseases like gastrointestinal disorders and gastro-intestinal infections. Apart from medicinal values, spider plant has been observed to have insecticidal, anti-feedant and pest repellent characteristics (Malonza et al., 1992). The bitter taste of spider plant is derived from polyphenolics, which constitute from 0.5% to 0.9% of the edible leaf (Chweya et al., 1997).

Systematic position of *Cleome gynandra* Linn. as per Bentham and Hooker classification.

Class	:	Dicotyledones
Sub class	:	Polypetalae
Series	:	Thalamiflorae
Order	:	Parietales
Family	:	Capparidaceae
Genus	:	*Cleome*
Species	:	*gynandra* Linn.

Materials and methods

Collection and identification of plant materials

Leaves of *Cleome gynandra* Linn. for the present study were collected during the summer and monsoon months of 2014 from Tiruchirappalli district, Tamil Nadu and care was taken to select healthy plants. The identity of the plant specimens was confirmed by the use of local Floras .The botanical identify was authenticated in the Department of Botany, Jamal Mohamed College, Tiruchirappalli. The collected leaf materials were washed thoroughly with tap water and dried under shade for ten days. Dried leaf material was grounded to coarse powder and stored in airtight container. It was then extracted with ethanol. The dry powder of sample was observed under U.V. light to evaluate the fluorescence. Chemical tests were performed on ethanol extract.

Qualitative analysis

Preparation of ethanol extract: About 600 g of powered material was taken in a clean, flat-bottomed glass container and soaked in 800 ml of 95% ethanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper. The filtrate thus obtained was concentrated using a rotary evaporator to get the extract.

Phytochemical screening

The plant extract was subjected to qualitative tests for the identification of the phytoconstituents present in it viz, alkaloids, carbohydrates, glycosides, phytosterols, fixed oils and fats, phenolic compounds & tannins, proteins and free amino acids, flavonoids, lignins and saponins (Harborne, 1998; Sofowora, 1982; Trease and Evans, 1989; Chase and Pratt, 1949).

Test for phenolic compounds

Two to three drops of 1% ferric chloride (FeCl3) solution was added into 2ml of extract. Phenolic compounds produce a deep violet colour with ferric ions.

Test for flavonoids

Shinoda test: To the alcoholic solution of the extract a few fragments of magnesium ribbon were added. To this concentrated hydrochloric acid (HCl) was added drop wise. Magenta colour was produced after few minutes which are the characteristic reaction of flavonoids.

Test for anthrquinones

5ml extract was boiled with 10ml of sulphuric acid and filtered while hot. The filtrate was shaken with 5ml of chloroform the chloroform layer was pipette out into another test tube then 1ml of dilute ammonia is added. The resulting solution was observed for colour changes. The change in colour indicates the presence of anthraquinones.

Test for tannins

Ferric chloride test: Water extract was treated with 15% ferric chloride test solution. The resultant colour was noted. A blue colour indicates condensed tannins; a green colour indicated hydrolysable tannins.

Test for saponins

The extract was diluted with 20ml of distilled water and it was agitated on a graduated cylinder for 15 min. the presence of saponins was indicated by the formation of 1 cm layer of foam.

Test for alkaloids

Mayer's test: The extract was dissolved in chloroform. The chloroform was evaporated and the residue was acidified and added few drops of Mayer's reagent (Potassium Mercuric Iodide). Alkaloids are precipitated by Mayer's reagent to give a cream coloured precipitate.

Wagner's test: The extract was dissolved in chloroform. The chloroform layer was evaporated, to the residue was acidified and added few drops of Wagner's reagent (Iodine in Potassium Iodide). Orange precipitate indicates alkaloids.

Test for the carbohydrate

The aqueous extract 5 ml was treated with the reagent of the starch (iodine). Any shift to blue violet indicates the presence of starch.

Test for glycosides

Fehling's test for reducing sugars (in glycosides): The extract was re-dissolved in water on the water bath. To 2 ml of the solution, in the test tube was added, 1ml each of Fehling's solutions A and B. The mixture was shaken and heated in a water bath for 10min. The colour obtained was recorded. A brick-red precipitate indicates reducing sugar.

Test for proteins

Xanthoproteic test: Extract was treated with few drops of concentrated HNO_3. Formation of yellow colour indicates the presence of proteins.

Test for steroids

For testing the presence of steroids 1ml extract was dissolved in 10ml of chloroform and equal volume of concentrated sulphuric acid was added from the walls of the test tube. Appearance of red colour in the upper layer and yellow with green fluorescence indicates the presence of steroids

Test for phytosterols

Liebermann-Burchard's test: One gram of the extract was treated with chloroform and filtered. The filtrate was treated with few drops of acetic anhydride boiled and cooled, concentrated sulphuric acid was added through the sides of the test tube. The formation of brown coloured ring at the junction of two liquids confirmed the presence of steroids.

Test for terpenoids

One ml of the extract was dissolved in 1ml of chloroform; 1ml of acetic anhydride was added following the addition of 2ml of concentrated sulphuric acid. Formation of reddish colour indicates the presence of terpenoids.

Test for fixed oil and fats

Press the extract in between the two filter papers, a permanent stain indicates the presence of fixed oil. Extract was treated with few drops of 0.5N potassium hydroxide and few drops of phenolphthalein and heat, formation of soap indicates the presence of fixed oil and fats.

Fluorescence analysis

Fluorescence analysis of the plant powder was observed in daylight and UV light (254nm) in a UV chamber (Kokoshi et al., 1958; Harborne ,1973) after treating with different chemical reagents is reported (Table 1). It can be used as a diagnostic tool for testing the adulterations.

Quantitative study of phytochemicals

Determination of alkaloid: Five grams of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Bohm and Kocipai Abyazan, 1994).

Determination of flavonoids: Ten grams of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper. The filtrate was later transferred into a crucible and

evaporated into dryness over a water bath and weighed to a constant weight (Obadoni and Ochuko, 2001).

Determination of saponin: Twenty grams of each grounded sample was put into a conical flask and 100cm3 of 20% aqueous ethanol was added. Then the flask was heated on a hot water bath for 4 h. with constant stirring at about 55°C. The mixture was then filtered and the residue was again extracted with another 200 ml 20% ethanol. The combined extract was reduced to 40 ml on a hot water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel, added20 ml diethyl ether in it followed by vigorous shaking. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml 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 oven, weighed and saponin content was calculated as percentage (Zafar and Lalwani, 1989).

Determination of glycosides:

Test 1: Extract of 200mg of the sample was taken warmed in a test tube with 5ml of diluted 10% sulphuric acid on the water bath at 1000 c for 2 minutes. Centrifuged; pipette off the supernatant. The acid extract was neutralized with 5% solution of NaOH. Added 0.1 ml of Fehling's solution A and then Fehling's solution B until alkaline (tested with pH paper) and heated on the water bath for 2 min. Noted the quality of red precipitate formed and compared with that formed in Test 2.

Test 2: Extract of 200mg of the sample was taken and 5ml of water was added instead of sulphuric acid. After boiling a volume of water was added equivalent to the volume of NaOH used in the Test 1, step 2 and above. 0.1 ml of Fehling's solution B was added until alkaline and heated on the water bath for 2 min. Note the quantity of red precipitate formed (Test 2). Compared the quality of the precipitate formed in test 2 that formed in Test 1. The precipitate in Test 1 was greater than that in Test 2. That indicated the presence of glycoside in the crude drug (Rai et al., 2008).

Determination of lignins: Two grams of the oven-dry material passed through an 80-mesh sieve were extracted in a Soxhlet apparatus with 200ml, 95% alcohol for 4 hours. The extracted residue was transferred to a round-bottomed flask of 300ml capacity and extracted with 150ml boiling water under a reflux for 1 hour. The contents of the flask are then filtered and residue was transferred back to the flask and hydrolyzed with 150ml. 5% H one hour. The hydrolyzed product was then collected in a weighed sintered glass crucible after being washed free of acid. The residue is weighed and well powdered. 0-2g lots were weighed out into 1000 ml beakers and treated with 20ml 72% H_2SO_4. The powder was well mixed with the acid and allowed to stand overnight, at a temperature varying between 20 and 220 (this was the range between 4p.m and 8a.m the following morning). The following morning the contents of the beakers were made up to 800ml. with water and boiled for 2 hours, the volume being maintained by occasional addition of water. Leave the beakers overnight to settle down the Lignin's, thereby facilitating filtration. The filtration was done under suction in crucibles with Whatmann filter paper. After drying, the precipitates were weighed and ignited and the lignin's calculated on an ash-free basis.

Determination of phytosteroids: One gram of powdered dried sample was extracted 3 times using a vortex mixture (15 min) with 7.5 ml chloroform. All the extracts were combined and evaporated to dryness. This chloroform extract contains free sterols and terpernoids. The residue was hydrolyzed with 2N HCl in methanol (2hr, 75-80ºC), neutralized with 10N NaOH, then diluted with 25ml water, and then the steroidal alkaloids and saponins were extracted 3 times with 10ml chloroform. The chloroform phase was collected and evaporated to dryness. The amounts of phytosteroids were weighed.

Determination of fixed oil: Transfer a 50g of the air dried, crushed drug to an extraction thimble, extract with Solvent ether in a continuous Soxhlet extraction apparatus for 6 h. Filter the extract quantitatively into a tarred evaporating dish and evaporate off the solvent on a water bath. Dry the residue at 105ºC to constant weight. Calculate the percentage of ether-soluble extractive with reference to the air-dried drug.

Results and discussion

The result of fluorescence analysis, qualitative and quantitative analysis of *Cleome gynandra* have been presented and discussed herewith. Fluorescence analysis of drug showed in Table 1 is an important parameter in detecting adulteration or improper

handling of drugs. It can serve as a valuable source of information and provide appropriate standards to establish the quality of this plant material in future study or application.

Table 1. Fluorescence analysis of *Cleome gynandra* in day light and in UV light.

S.No.	Chemical test	Day light	UV light
1	Sample	yellow	Dark yellow
2	Powder + 1N aq. NaOH	yellow	Green
3	Powder+ 1N alc. NaOH	Yellowish green	Green
4	Powder + 1N HCl	Greenish yellow	Dark green
5	Powder + 50% HNO_3	Dark green	Light Green
6	Powder + 50% H_2SO_4	Yellow	Green
7	Powder + Methanol	Green	Light green
8	Powder + NH_3	White	Green
9	Powder + I_2	Reddish orange	Yellowish brown
10	Powder + $FeCl_3$	Brownish orange	Light brown

The phytochemical analysis of leaf extracts of *Cleome gynandra* were tested by different specific tests. Methanol, ethanol, petroleum ether, chloroform and acetone leaf extracts of *Cleome gynandra* L., were analyzed for their compounds such as tannins, saponins, flavonoids, steroid, cardiac glycosides, alkaloids and presented in Table 2. Phytochemical analysis table explained that methanol and ethanol extracts showed more phytochemicals than acetone, petroleum ether and chloroform. Phytochemicals like tannins, phenols, flavonoids, cardiac glycosides, steroids were found to present in all the tested extracts.

On the other hand saponins are found in methanol, ethanol and acetone they were not found in petroleum ether and chloroform. Terpenoids and anthraquinones were not found in all the tested extracts. Whereas alkaloids are present in all the extracts but absent in chloroform. Tannins are present in all the extracts but volatile oils are absent. Earlier phytochemical studies also revealed that *Cleome gynandra* has contains several constituents like carotenoids, cardiac glycosides, cyanogenic glycosides, flavanoids, saponins, triterpenes, sugars and tannins (Anbazhagi et al., 2009).

Table 2. Phytochemical screening of the leaf extracts of *Cleome gynandra* in various solvents.

S.No.	Phytochemical constituents	Methanol	Ethanol	Chloroform	Petroleum ether	Acetone
1	Alkaloids	+	+	-	+	+
2	Carbohydrates	+	+	-	-	-
3	Glycosides	+	+	+	+	+
4	Saponins	+	+	-	-	+
5	Tannins	+	+	+	+	+
6	Phytosterols	+	+	-	-	+
7	Proteins	+	+	-	-	-
8	Flavonoids	+	+	+	+	+
9	Lignin	+	+	-	-	-
10	Volatile oils	-	-	-	-	-
11	Fixed Oils and Fats	+	+	-	-	-
12	Phenols	+	+	+	+	+
13	Anthraquinines	-	-	-	-	-
14	Terpenoids	-	-	-	-	-
15	Steroids	+	+	+	+	+
(+)- Presence (-) - Absence						

The phenolic compounds are considered as being a major group to the number of the secondary metabolites that contributes to the antioxidant activity of the plant. The presence of phenolic compounds in the plant indicates that this plant may have the ability as an anti-microbial agent (Shivhare et al., 2010). It has been reported that most active principles in plants are frequently flavonoids, steroids, glycosides and alkaloids.

These phytoconstituents may be responsible for the many pharmacological actions of the plant like wound healing (Sharmila et al., 2007), cholesterol lowering and antidiabetic activity (Delanty and Dichter, 2000). Phytosterols are an important breakthrough in the human fight against high cholesterol. It has been known that plant steroids, flavonoids are antioxidants. These antioxidants are compounds that reduce the formation of free radicals or react with and neutralize them thus potentially protecting the cell from oxidative damage. The results of quantitative phytochemicals like total alkaloids, glycosides, saponins, phytosterols, total flavonoids, lignin and fixed oil are given in Table 3.Except for total alkaloids and total flavonoids, all other constituents were found to be lesser in amount.

Table 3. Quantitative phytochemical evaluation of *Cleome gynandra.*

S.No.	Phytochemical constituent	Content
1	Total alkaloids (mg/kg)	1.76
2	Glycosides (mg/kg)	0.26
3	Saponins (mg/kg)	0.14
4	Phytosterols (mg/kg)	0.03
5	Total Flavonoids (mg/kg)	0.76
6	Lignin (mg/kg)	0.01
7	Fixed Oils(µg/lit)	0.08

The results of the micronutrient analysis of ethanol extract of *Cleome gynandra* showed the presence of carbohydrates, fats, proteins, reducing sugars and fixed oil (Table 4). The macronutrients; proteins, carbohydrate and reducing sugar may be involved in the energy providing and body building function of this plant.

Table 4. Results of the macronutrient analysis of ethanol extract of *Cleome gynandra.*

S.No.	Constituents	Presence/ Absence
1	Total sugars	+
2	Reducing sugars	+
3	Total proteins	+
4	Fixed oils and fats	+

Conclusion

The plant phytochemical studies could be provide an answer to the society seeking for better therapeutic medicine from natural sources which is supposed to be more efficient with less or no side effects when compared to the commonly used synthetic chemotherapeutic agents. The results obtained from the present phytochemical analysis of the leaves of *Cleome gynandra* showed the presence of alkaloids, tannins, flavonoids, cardiac glycosides, steroids, saponins, phenols, flavonoids, phytosterols, lignins, and macronutrient analysis revealed the presence of proteins, carbohydrates, reducing sugar, fats and oil. Alkaloids and flavonoids have been used as antiviral, antibacterial, antiamoebial and anticancer agents. Phenols and flavonoids are the groups of secondary metabolites are of great importance as cellular support material because they form the integral part of cell wall structure by polymeric phenolics and they can protect the human body from the oxidative stress which may cause many disease, including cancer, cardiovascular problems and ageing .This study justifies the use of *Cleome gynandra* in the treatment of many ailments like diabetic mellitus, carcinoma, heart disease, asthma and rheumatism. Non-nutrient phytochemical content which act as antioxidant against dangerous free radicals in the body system. In this study suggest the identified phytochemical compounds may be the bioactive constituents which are medicinally valuable. Therefore, extracts from these could be seen as a good source for useful drugs. The present study concludes that the leaves of *Cleome gynandra* can be utilized as an alternative source of useful drugs.

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