



PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF METHANOLIC LEAF EXTRACT OF *Cleome viscosa* L.

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Abstract

Cleome viscosa is an annual sticky herb belongs to the family of Capparidaceae and it is commonly known as "Wild mustard". In traditional systems of medicine, the plant was reported to possess beneficial effects as an antihelmintic, antiseptic, carminative, antiscorbutic, febrifuge, anticancer and cardiac stimulant. In the present study, the phytochemical analysis and GC-MS analysis of *Cleome viscosa* leaf parts was conducted and the presence of various plant metabolites including alkaloids, flavanoids, glycosides, tannins saponinns, steroids, terpenoides and phenolic compounds was analyzed. The GC-MS results were noticed in the methanolic extract of *Cleome viscosa* was reported with molecular weight, retention time, peak area and molecular formula. The compounds were identified by comparing their retention time and peak area with that of literature and by interpretation of mass spectra. The plant *Cleome viscosa* was used in various industries and its applications includes wound healing, antioxidant, antiinflammatory, antimicrobial and cancer preventive.

Key words: *Cleome viscosa*, GC-MS analysis, Antioxidant, Retention time, Anticancer, Antiscorbutic and Methanolic extract.

1. Introduction

Medicinal plants have provided the modern medicine with numerous plant derived therapeutic agents. Plants have potential uses especially as traditional medicine and pharmacopeial drugs. A large proportion of the world population depends on traditional medicine because of the scarcity and high costs of orthodox medicine. Many plants contain a variety of phytopharmaceuticals, which have found very important applications in the fields of agriculture, human and veterinary medicine. Natural products play a dominant role in the development of novel drug leads for the treatment and prevention of diseases. Knowledge of the chemical constituents of plant is helpful in the discovery of therapeutic agent as well as new

sources of economic materials like oil and gums (Peter *et al.*, 2012). Medicinal plants are the basis of modern pharmaceuticals used today for various ailments, as it has a good source of natural antioxidants for medicinal use and related to radical mechanism (Gayathri *et al.*, 2013).

Medicinal plants are expensive gift from human to nature. The approval of traditional medicine as an alternative form of health care and the improvement of microbial resistance to the existing antibiotics has lead researchers to scrutinize the antimicrobial compounds. Herbal medicines are safer than synthetic medicines because the phytochemicals in the plant extract target the biochemical pathway. Medicinal plants have been used all over the world for the treatment and prevention of various ailments, particularly in developing countries where infectious disease is

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endemic and modern health facilities and services are inadequate. The tribal communities of many countries are still using medicinal plants to cure sickness (Balaji *et al.*, 2014)

Phytochemicals are compounds that are produced by plants "phyto" means "plant". They are found in fruits, vegetables, grains, beans, and other plants. Some of these phytochemicals are believed to protect cells from damage that could lead to cancer. Some scientists think that you could reduce your cancer risk by as much as 40 % by eating more vegetables, fruits, and other plant foods that have certain phytochemicals in them. Plants, the most wonderful gift from nature have been used as an origin of drugs. Various types of drugs are obtained from them. These types of plants are known as medicinal plants. We use one or more of its organ for therapeutic purpose as a precursor of synthesizing of many useful drugs. According to some generous estimates, almost 80 % of the present day medicines are directly or indirectly obtained from plants (Amir *et al.*, 2014).

Gas Chromatography–Mass Spectrometry (GC-MS) is a hyphenated analytical technique that combines the separation properties of Gas-liquid chromatography with the detection feature of mass spectrometry to identify different substances within a test sample. GC-MS is used to separate the volatile, non-ionic and thermally stable substitutes in a sample. GC-MS commonly uses electron impact and chemical ionization technique. GC-MS is mainly used for the analysis of esters, fatty acids, alcohols, aldehydes and terpenes. GC-MS is exclusively used in bioanalysis of blood, urine for the presence of barbiturates, narcotics, alcohols, residual solvents, drugs like anesthetics, anticonvulsant, antihistamine, antiepileptic drug, sedative hypnotics, narcotics and food items. GC-MS is widely used in pharmaceutical industries for analytical research and development, quality control, quality assurance, production, pilot plants departments for active pharmaceutical ingredients (API), bulk drugs and formulations. GC-MS is used in industries for the analysis of aromatic solvents, inorganic gases, amino alcohol in water,

impurities in styrene, glycol, diols, xylene and allergens in cosmetics (Ashish *et al.*, 2014)

Cleome viscosa leaves and young shoots used to cook like a vegetable, which is having sharp mustard like flavour. The analgesic, antimicrobial, antidiarrhoeal, antipyretic, hepatoprotective, antihyper lipidemic and antiulcer activities. The popular use of the whole plant and leaves refers mainly to its antiseptic, anti inflammatory activity and wound healing (Panduraju *et al.*, 2011). The leaves are diaphoretic, rubefacient and vesicant and are used as an external application to wounds and ulcers. The juice of the leaves has been used to relieve earache. Leaf decoction is used in the treatment of respiratory tract infections, wounds and jaundice. Plant extracts are also used to kill insects and parasitic nematodes. The flavonoid glycoside isolated from *Cleome viscosa* showed significant antiinflammatory activity. This antiinflammatory effect of the flavonoid glycoside may be due to the inhibition of prostaglandin synthesis (Upadhyay, 2015). *Cleome viscosa* leaves possess high phenolic and flavonoid contents which show potential antioxidant activity and free radical scavenging activity. Both leaf and seed oil is used for various veterinary and medicinal purposes while whole plant is used to cure rheumatism (Ravi *et al.*, 2015). The present study aimed to study the phytochemical screening and GC-MS analysis of methanolic leaf extract of *Cleome viscosa*.

2. Materials and Methods

Collection of plant materials

The leaves of *Cleome viscosa* leaves were collected from Elagiri village, Vellore district, Tamil Nadu, India.

Preparation of plant extract

Leaves of *Cleome viscosa* (Twenty gram) was dried and powdered using Morter and Pestle. The plant powder was extracted with methanol solvent for 6 hours using Soxhlet apparatus. After the extraction process, the extract was filtered



through the Whattmann No.1 filter paper in a beaker. The methanolic extract was stored at room temperature for further use.

Phytochemical screening

Phytochemical tests were carried out using methanolic extract *Cleome viscosa* leaves such as follows:

Test for Alkaloids

Small amount of solvent free extract was dissolved in Dil. HCl and then 1.2 ml of the solvent extract was mixed with 0.1 ml of Mayer's reagent. Formation of white precipitate shows the presence of alkaloids (Evans,1997).

Test for Flavonoids

A pinch of the extract was dissolved in 5 ml of distilled water. Ten per cent of sodium hydroxide was prepared (10 g in 100 ml water) and mixed with the extract. Formation of yellow colour which disappears by the addition of Dil. HCl shows the presence of flavonoids (Trease and Evans, 2002).

Test for Glycosides - Liebermann's Test

Two ml of the sample was dissolved in 2 ml of chloroform and then 2 ml of acetic acid was added on it. The solution was cooled well in ice. Sulphuric acid was then added carefully. A colour change from violet to blue to green indicates the presence of a steroidal nucleus (Trease and Evans, 2002).

Test for Phenol

The mixture was prepared dissolving 0.1 mg of sample in 1 ml of methanol. From the mixture, 20 µl was taken and 180 µl of water was added. Then, 0.5 ml of Folin phenol reagent, 0.5 ml of water 1 ml of 7.5 % sodium carbonate was added to the mixture. Then, it was kept 2 hours for incubation and the absorbance was read at 726 nm by spectrophotometer. Gallic acid was used as phenol standard and expressed as Gallic acid equivalent (Trease and Evans, 2002).

Test for Saponins

A pinch of the extract was dissolved in 1 ml of distilled water. It was warmed in the heating mantle for 2 minutes at 60 °C. Then, 0.5 ml of distilled water was added to it and shaken well. Appearance of froth on the top layer shows the presence of saponins (Sofowara,1993).

Test for Steroids

A red colour produced in the lower chloroform layer when 2 ml of organic extract was dissolved in 2 ml of chloroform and 2 ml concentrated sulphuric acid was added in it and indicates the presence of steroids. Development of a greenish colour when 2 ml of the organic extract was dissolved in 2 ml of chloroform and treated with sulphuric and acetic acid indicates the presence of steroids (Sofowara, 1993).

Test for Terpenoids

A small amount of extract was dissolved in 1 ml of chloroform and 1 ml of Concentrated sulphuric acid. Formation of reddish brown coloration confirms the presence of Terpenoids (Sofowara,1993).

Test for Tannins

About 2 ml of the sample was stirred with 2 ml of distilled water and few drops of FeCl₃ solution were added. Formation of green precipitate was indication of presence of tannins (Trease and Evans, 2002).

Gas Chromatography - Mass Spectroscopy (GC-MS) Analysis

The GC-MS analysis of *Cleome viscosa* powder leaves extract with in absolute alcohol was performed using a Clarus 500 Perkin Elmer Gas Chromatography equipped with a Elite - 5 capillary column (5 % phenyl 95 % dimethyl polysiloxane) (30 nm X 0.25 mm ID X 0.25 µm df) and mass detector turbomass gold of the company which was operated in EI mode. Helium was the carriers gas at a flow rate of 1 ml/min and the injector was operated at 290 °C and the oven



temperature was programmed as follows; 50 °C at 8 °C/min to 200 °C (5 min) at 7 °C/min to 290 °C (10 min) (Peter *et al.*, 2012).

4. Results and Discussion

Phytochemical analysis of methanolic extract of *Cleome viscosa* leaves

The qualitative phytochemical analysis of the raw materials, methanolic extracts was chemically tested for phytochemical constituents using standard procedures recommended by Sofora (1994) and Trease and Evans (1989). The phytochemical tests are carried out include alkaloids, flavonoids, tannins, terpenoids, saponins, phenols, steroids and glycosides. The leaves are diaphoretic, rubefacient and vesicant and are used as an external application to wounds and ulcers. The juice of the leaves has been used to relieve earache. Leaf decoction is used in the treatment of respiratory tract infections, wounds and jaundice. Plant extracts are also used to kill insects and parasitic nematodes. The flavonoid glycoside isolated from *Cleome viscosa* showed significant antiinflammatory activity. This antiinflammatory effect of the flavonoid glycoside may be due to the inhibition of prostaglandin synthesis (Upadhyay, 2015).

Preliminary phytochemical analysis of methanolic extract of *Cleome viscosa* leaves

Plants are rich and useful source of primary and secondary metabolites like proteins, lipids, carbohydrates, alkaloids, flavonoids, terpenoids, tannins, etc. These metabolites are useful for the plants as well as for the human being for the treatment of various illnesses. Determinations of these metabolites are helpful to know the medicinal as well as food value of respective plants (Kumar *et al.*, 2013).

In the present investigation, the qualitative analysis was carried out by methanolic extract of *Cleome viscosa* leaves. The extracts showed the presence of phytochemical constituents namely, alkaloids, flavonoids, glycosides, phenolic compounds, saponins, steroids and tannins.

Terpenoids was absent in the methanolic extract of *Cleome viscosa* leaves (Table – 1).

Table – 1: Preliminary phytochemical screening of *Cleome viscosa* leaves

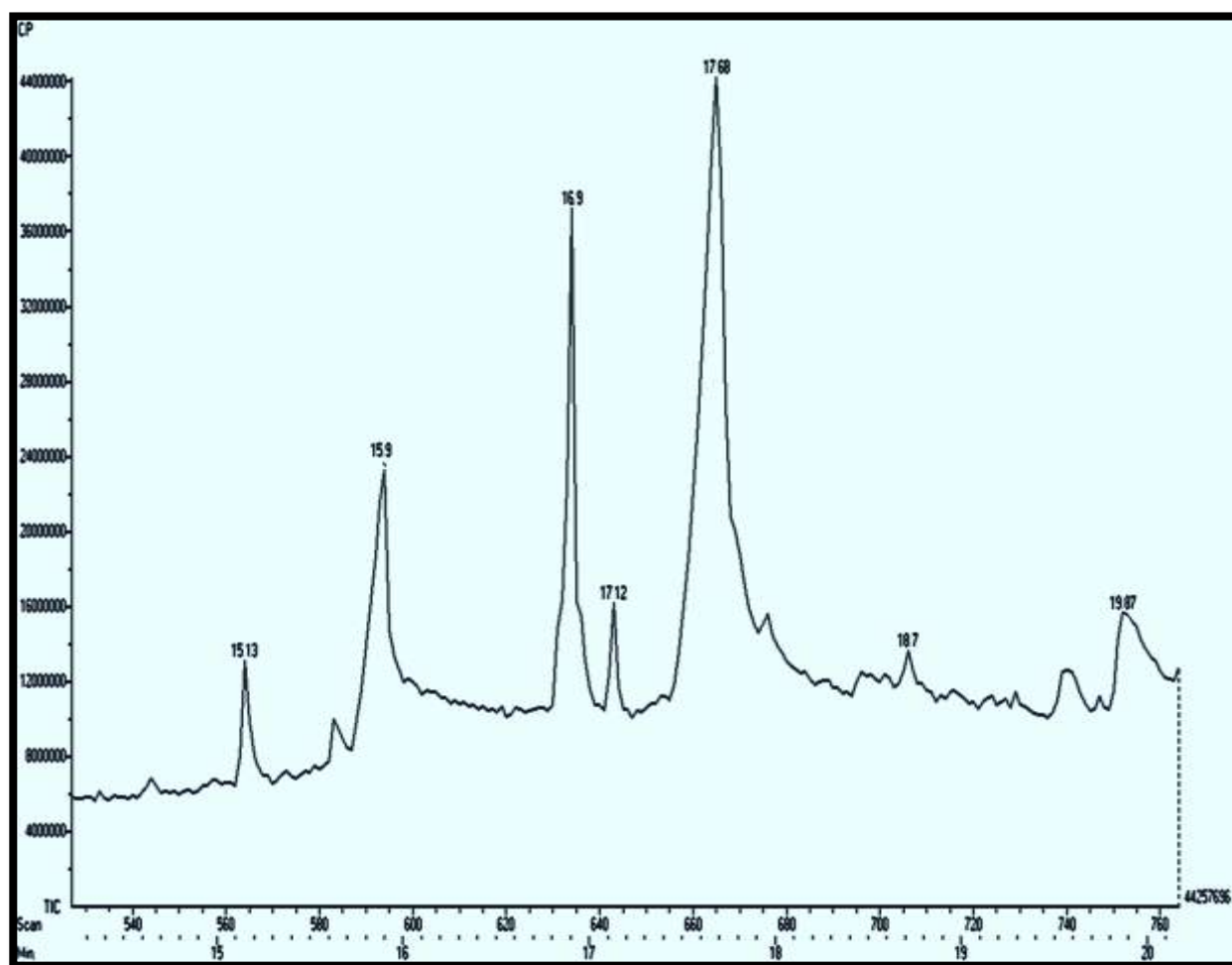
Phytochemical constituents	Methanolic extract of <i>Cleome viscosa</i> leaves
Alkaloids	+
Flavonoids	+
Glycosides	+
Phenol	+
Saponins	+
Steroids	+
Tannins	+
Terpenoids	-

+ Present, - Absent

Gas Chromatography - Mass Spectroscopy (GC-MS)

The GC - MS analysis plays an important role in the analysis of major unknown components of methanolic extract of *Cleome viscosa* leaves. This analysis was reported the presence of seven components was employed the retention time, molecular weight, molecular formula and peak area. Paul John Peter (2012) reported that the GC-MS analysis, were identified in leaf powder of *Stylosanthes fruticosa*. The 33 compounds predominantly phenolic compounds and flavonoids derivatives are present included, carbohydrate and glycoside, saponin and phytosterols compounds. Protein and alkaloids was limited in the leaf extract. The seven phytochemical constituents present in methanolic extract of *Cleome viscosa* leaves is identified by using the GC-MS method was listed in Table – 2. The leaves of *Cleome viscosa* which are mostly used as a source of medication in traditional medicines was considered to examine the properties of the plant. A wide variety of phytoprinciples are present in *Cleome viscosa* (Mali, 2010). The results revealed that *Cleome viscosa* contained alkaloids, tannins, saponins and flavonoids. These phytochemicals may have contributed for antimicrobial activity of *Cleome viscosa* against Oitis media pathogens. This result



Figure - 1: GC-MS chromatogram of *Cleome viscosa* leavesTable – 2: Phytochemical constituents of methanolic extract of *Cleome viscosa* leaves by GC - MS analysis

S. No	Retention time (RT)	Name of the compounds	Molecular weight	Molecular formula	Peak area (%)
1	16.9	Ethyl oleate	310	$C_{20}H_{38}O_2$	4.60%
2	18.7	Cholestan-3-ol, 2-methelene	386	$C_{27}H_{46}O$	1.45%
3	15.13	1[+]-Ascorbic acid 2,6-dihexadecanoate	652	$C_{38}H_{68}O_8$	1.31%
4	15.9	D-Fructose diethyl mercaptal penta acetate	496	$C_{20}H_{32}O_{10}S_2$	2.32%
5	17.12	Pregn-5-en-20-one-3[acetyloxy]-cyclic 20-[1-2-ethanediyl acetal], [3a], [5a]	434	$C_{25}H_{38}O_2S_2$	2.52%
6	17.68	[2, 5, furandione, dihydro-3-octadecyl	352	$C_{22}H_{40}O_3$	4.42%
7	19.87	Pyrrolo [1, 2, a] pyroline 1, 4 - hexahydro, 3-[phenyl methyl] dione, hexahydro, 3-[phenyl methyl]	244	$C_{14}H_{16}N_2O_2$	6.54%



is in analogy with previous reports of Koche *et al.* (2010) who reported presence of alkaloids, flavonoids, tannins, saponins and terpenoids in the leaves of *Cleome viscosa*. The leaves of *Cleome viscosa* which are mostly used as a source of medication in traditional medicines was considered to examine the properties of the plant. A wide variety of phytoprinciples are present in *Cleome viscosa* (Sangitalavate *et al.*, 2010). The results revealed that *Cleome viscosa* contained alkaloids, tannins, saponins and flavonoids. The GC-MS analysis of the methanolic extract of *Cleome viscosa* leaves are showed the bioactive components are found to the, 1) Ethylolate, 2) Cholestan-3-ol, 2-methelene, 3) 1[+]-Ascorbic acid 2, 6 hexadecanoat, 4) D - Fructose diethyl mercaptalpentaae, 5) Pregn-5en-20-one-3[acetyloxy] - cyclic 20-[1-2 ethane diylacetal], [3a], [5a], 6) 2, 5, furandione, dihydro - 3 - octadecyl, 7) Pyrrolo [1, 2, a] pyrozone 1, 4 - dione, hexahydro, 3 -phenyl methyl.

4. Conclusion

From this present research, it was concluded that the methanolic extract of leaves of *Cleome viscosa* leaves possess various potential bioactive compound and is recommended as a plant of phytopharmaceutical importance and explore the potential compounds responsible for the biological activity. Methanolic extract of *Cleome viscosa* leaves and GC-MS analysis of bioactive components suggest that the pharmacological activity and anticancer activity should be evaluated.

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