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Antimicrobial Activity, Phytochemical Analysis and HPLC Screening Of *Cleome viscosa.* L

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Abstract

The study was aimed to investigating the antimicrobial screening of ethanolic extracts of *Cleome viscose* against pathogenic bacteria and fungi responsible for common infections. The present investigation may be concluded that the plant *Cleome viscosa* is endowed with significant antimicrobial due to the presence of active constituents, there by justifying its use in the indigenous system of medicine.The seeds of *Cleome viscosa* are used in traditional systems of medicine for the treatment of many diseases in Asia. The present research work was conducted to investigate the invitro antimicrobial activity of seed extracts of plant *Cleome viscosa.* The ethanol extract of seeds of plants were screened against microbial species. The test materials have showed the significant antimicrobial activity. The zone of inhibition is found to be (12mm to 23 mm) bacteria and fungi. The present research work was identified phytochemical test presence of active biomolecules such as Alkaloid, tannins flavonoids, Fixed oil and fats, Gum and mucilage, sterols were present in water extracts of *Cleome viscosa.* To perform the solvent extraction and evaluate the Infra-Red and HPLC analysis.

Keywords: *Cleome viscosa* seed, column chromatography, anti-microbial activity, phytochemical analysis, Infra- Red, HPLC.

Introduction

Since ancient times, plants have been source of crude material for medicines. A rich heritage of knowledge of medicinal importance of plants is available in India amongst the common people. Generally, it is estimated that more than 3,500 plant species in India are useful as a source of crude drug. Near about 2,500 plants are ethno medicinally important. There are too many plant

species which are considered as a weed, but they are also medicinally important. The products obtained from plants are used in various traditional and modern methods of therapy. The trend of using natural products as a medicine is ever increasing. The invention of modern biotechnological and bioinformatics techniques have helped new drug discoveries.

Cleome viscosa L. is a widely distributed sticky herb with yellow flowers and long slender pods containing seeds, which similar those of mustard (Hindi), Hurhuria (Bengali), Nayikkadugu (Tamil) in South Asian folk medicine, found throughout the larger part of Indian Subcontinent, often in waste places. The plant finds its use in the traditional system of local medicine as a laxative and diuretic. It is reported to be useful in the treatment of malarial fevers, fever due to indigestion, skin diseases, leprosy, blood diseases, and uterine complaints. In the Unani system of medicine, the seeds of the plant are documented as anthelmintic and detergent, and are given to treat fever and diarrhoea. The seeds are used for anthelmintic while the leaves are useful for healing wounds.

In Ayurveda system of medicine, in the plant is used in fever, inflammation, liver diseases and diarrhoea. The rural people use the fresh juice of the crushed seed for infantile convulsions and mental disorder. The juice of the plant diluted with water is given internally in small quantities in fever and the seeds and leaves are useful in wound healing. The object of present study is to evaluate various pharmacognostical parameters such as microscopy, physiochemical parameters, and phytochemical studies of the plant. Antibacterial and antifungal activity to separate the different compounds from the seed at different time interval. To analyse the antibacterial and antifungal activities of three compound and analyse the phytochemical compound, Infra-red, and HPLC analysis of the plant.

Materials and Methods

PLANT SAMPLE COLLECTION

The plant samples were collected from Orathanadu, Thanjavur District. The seed were separated from the collected plant and dried under shade. After drying, it was powdered and used for our studies.

COLUMN CHROMATOGRAPHY

Column chromatography is used to purify liquids by separating an organic solvent from a mixture of solvent. The seed extract was prepared by grinding the mixture in mortar pistol containing 22 ml of acetone, 3ml petroleum ether and calcium carbonate. The pigments was filtered and mixed with 20 ml petroleum ether and 20ml of 10% aqueous sodium chloride solution. The separating funnel was shaken carefully and the lower layer was allowed to drain into the beaker. A plug of cotton is placed to the bottom of the column so that silica and soil won't fall out. Slurry of silica was prepared and poured into the column carefully. It is allowed to settle and sand is added. The sample was added using a pasture's pipette carefully above the sand. The eluent is added on top of the sand. The mobile phase slowly flows down through the silica gel column by gravity leaving behind zone of colour and a component was eluted from the column.

ANTI MICROBIAL ACTIVITY

This study involves measurement of relative activity of compounds present in extracts against growth of microorganisms under standard conditions. It was measured by using zone of inhibition. 10 Pus samples were collected from the patient and stored in the Ame's transport medium. These collected samples were containing mixed cultures of microorganisms. The ear micro flora of volunteers was obtained by using sterile cotton swab. Then the swabs were inoculated in sterile nutrient medium. The Muller Hinton Agar was used as a medium for bacterial cultures and Potato dextrose agar was used for fungal cultures. Then by using spread plate technique, microbial cultures in plate were developed [1,2]

PHYTOCHEMICAL ANALYSIS

The compounds that are responsible for therapeutic effect are usually the secondary metabolites. The preliminary phytochemical analysis was carried out to find out the Sterols, Flavonoids, Alkaloids, Tannins, Phenol, lignin, Fixed oil and fats, gum and mucilage.

IR SPECTRUM ANALYSIS

FTIR relies on the fact the most molecules absorb light in the Infra-red region of the electromagnetic spectrum this absorption corresponds specifically to the bonds present in the molecule. The frequency ranges are measured as wave numbers typically over the range 4000-400 cm-1.

HPLC ANALYSIS

High performance liquid chromatography (HPLC) is now one of the most powerful tools in analytical chemistry, with ability to separate identify and quantitative the compounds that are present in any sample that can be dissolved in a liquid. HPLC method for the analyse which gives the best separation of peaks. The band in the sample were obtained the purify compounds. Detection: under UV light 326nm.

Chi – Square test (X2)

In this study chi – square test (X^2) was applied (9). The purpose of chi – square test (X2) was to decide whether the set of observed data (Antibiogram of microorganisms) agrees with the standard antimicrobial disc susceptibility test (NCCLS, 2002).

Result and Discussion

The previous study antimicrobial activity of various extracts of seeds of *Cleome viscosa*was found very significant. The zones of inhibition obtained by extracts of were found against bacteria from 12 to 17 mm and against fungi are from 12 to 14 mm. The present study *Cleome viscosa* seed were collected [3] and analyse the activity of crude extract and compound. The collected seeds were extracted and showed the antimicrobial activity against the pathogen.

The zone of inhibition in the crude extract against *Klebsiella pneumoniae* (18mm), *E.*coli(16mm) *Bacillus subtilis*(16mm), *Staphylococcus aureus*(17mm) *Pseudomonas aeruginosa* (15mm)(Table.1) and fungus against *Mucor sp*(17mm*),* *Aspergillus sp*(14mm), *Penicillium sp*(15mm)(Table.2). After using the column chromatography the extract were collected at different time interval. The collected extracts were identified as the name (Compound-I, Compound-II and Compound-III). The antimicrobial activity against their three compounds. The compound II gave the maximum zone of inhibition. In bacteria *Klebsiella pneumoniae* (20mm), *E.coli(*19mm), *Bacillus subtilis*(18mm), *Staphylococcus aureus(*17mm), *Pseudomonas aeruginosa*(19mm)(Table.3) and fungus *Mucor sp(*20mm), *Aspergillus sp*(16mm), *Penicillium sp*(17mm)(Table.4) compared with crude extracts. The results conclude that the maximum zone of inhibition gave the gram negative bacteria *Klebsiella pnemoniae* (20mm), the maximum zone of inhibition gave the fungus *Mucor sp*(20mm). Table.5 the following Phytochemical compound were identified the presence of active biomolecules such as Alkaloid, tannins flavonoids,Fixed oil, fats, Gum and mucilage, sterols were present in water extracts of *Cleome viscosa* [4,5]. The functional groups were identified using FTIR. (Table.6 and Figure.1) [6]. HPLC analysis the compound separated using column chromatography showed the retention time of 2.610 (Table.7).The chi-square value showed Table 1 and Table 3, which was less that the calculated table value X2 (0.05) = 5.991 at 5% level of significance. The above result lead to the conclusion that the data is consistent with the hypothesis and diameter of zone of inhibition obtained from observed data showed similarities with experimental data[7].

TABLE: 1 ZONE OF INHIBITION FORMED BY CRUDE EXTRACT OF *Cleome viscosa* SEED POWDER AGAINST BACTERIA

S.NO	Antibacterial agent	Name of the bacteria	µg	Zone of inhibition in diameter		$X^2 = \frac{(O-E)^2}{E}$
				Standard value (mm)	Observed value (mm)	
1	*Cleome viscosa* seed powder	*Klebsiella pneumoniea*	60	21	18	0.428
2		*E.coli*	60	21	16	1.190
3		*Bacillus subtilis*	60	21	16	1.190
4		*Staphylococcus aureus*	60	21	17	0.761
5		*Pseudomonas aeruginosa*	60	21	15	1.714

Table 2. ZONE OF INHIBITION FORMED BY CRUDE EXTRACT OF *Cleome viscosa* SEED POWDER AGAINST FUNGUS

S.NO	Antifungal agent	Name of the fungus	µg	Zone of inhibition in diameter		$X^2 = \frac{(O-E)^2}{E}$
				Standard value (mm)	Observed value (mm)	
1	*Cleome viscosa* seed powder	*Aspergillus niger*	30	21	14	2.333
2		*Penicillium sp*	30	21	15	1.714
3		*Mucor sp*	30	21	17	0.761

Table 3. ZONE OF INHIBITION FORMED BY COMPOUND EXTRACT OF *Cleome viscosa* SEED POWDER AGAINST BACTERIA

S.NO	Antibacterial agent	Name of the bacteria	µg	Zone of inhibition in diameter		$X^2 = \frac{(O-E)^2}{E}$
				Standard value (mm)	Observed value (mm)	
1	*Cleome viscosa* seed powder	*Klebsiella pneumoniea*	60	21	20	0.047
2		*E.coli*	60	21	19	0.190
3		*Bacillus subtilis*	60	21	18	0.428
4		*Staphylococcus aureus*	60	21	17	0.761
5		*Pseudomonas aeruginosa*	60	21	19	1.190

Table 4. ZONE OF INHIBITION FORMED BY COMPOUND EXTRACT OF *Cleome viscosa* SEED POWDER AGAINST FUNGUS

S.NO	Antifungal agent	Name of the fungus	µg	Zone of inhibition in diameter		$X^2 = \frac{(O-E)^2}{E}$
				Standard value (mm)	Observed value (mm)	
1	*Cleome viscosa* seed powder	*Aspergillus niger*	30	21	16	1.190
2		*Penicillium sp*	30	21	17	0.761
3		*Mucor sp*	30	21	20	0.047

Table 5. PHYTOCHEMICAL ANALYSIS OF *Cleome viscosa*

S.NO	PHYTOCHEMICAL SCREENING	RESULT
1	Phenol	Negative
2	Flavonoid	Positive
3	Alkaloids	Positive
4	Glycosides	Negative
5	Tannins	Positive
6	Fixed oil and fats	Positive
7	Lignin	Negative
8	Carbohydrate	Positive
9	Steroids	Positive
10	Protein & Free amino acid	Negative
11	Gums and Mucilage	Positive
12	Saponins	Negative

Table 6.FT-IR spectrum is used to obtain the graph and the band stretching is interpreted as follows.

S. No	Peak Value	Stretching	Interpretation
1	420.48	C-Br stretching	Halogen
2	671.23	C-H stretching	Alkyl
3	794.67	C-H stretching	Alkanes
4	1020.34	C-O stretching	Alcohols
5	1112.93	C-H bending	Alkanes
6	1382.96	C-O stretching	Phenols
7	1442.75	C-H stretching	Alkanes
8	1525.69	C=C stretching	Aromatic group
9	1635.64	C=C stretching	Aromatic group
10	1654.92	C-C stretching	Alkenyl group
11	1683.64	C-O stretching	Amide group
12	1737.86	C-O stretching	Ester group
13	2854.65	C- H stretching	Alkyl group
14	2924.09	C-H stretching	Alkyl group

15	3446.79	O-H stretching	Alcohol group

Table. 7 HPLC ANALYSIS REPORT

Acquired by : Admin
Sample Name : Cleome –vis
Injection Volume : 20 µg
Data Filename : cleome-vis.lcd
Method Filename : Bdu-Meth.lem

Detector A ChI 326nm **Peak Table**

Peak	Ret.Time	Area	Height	Area%	Height%
1	2.610	10880	446	100.000	100.000
Total		10880	446	100.000	100.000

Fig1. FT-IR spectrum is used to obtain the graph

Conclusion

The present investigation found that *Cleome viscosa* possesses notable antimicrobial properties, shows this seed used to recover the problem of Skin diseases. The human beings infected for ear ache. The continuous uptake of juice to remove pus from ear.

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