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RESEARCH ARTICLE

Evaluation of *in vitro* Antioxidant, Antibacterial and Anticancer activities of leaf extracts of *Cleome rutidosperma*

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ABSTRACT:
The present study was aimed to analyze the phytochemicals of leaf extracts of *Cleome rutidosperma* and to check their antioxidant, antimicrobial and anticancer activities using *in vitro* methods. The hexane, ethyl acetate and methanol extracts of *C. rutidosperma* were extracted using 70%v/v of above mentioned solvents using Rotary evaporator. The phyto-constituents such as alkaloids, flavonoids, tannins, saponins, steroids, proteins and polyphenols were detected using standard chemical methods. The ability of plant extracts to scavenge the stable free radical DPPH was analyzed. The IC_{50} values of each extracts were determined using the standard graphs. Antibacterial activity of the prepared extracts was performed against *Bacillus cereus*, *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi. In vitro* cytotoxicity of methanol extract of *C. rutidosperma* was determined using MTT (3-(4,5-Dimethylthiazol-2-Yl)-2, 5-Diphenyltetrazolium Bromide) assay. Among the hexane, ethyl acetate and methanol extracts of leaves of *C. rutidosperma*, the methanol extract showed positive for all phytochemicals tested in this study except saponins. At a concentration of 150µg/ml the methanol extract showed the zone of inhibition of 12mm against both *B. cereus* and *E. coli*, ethyl acetate showed 10mm against *S. aureus* and 12mm against *S. typhi*. The methanol extract also exhibited significant antioxidant and anticancer activities. This study reveals that the plant *Cleome rutidosperma* possesses medicinal properties like antibacterial, antioxidant and anticancer activities.

KEYWORDS: *Cleome rutidosperma,* Hexane, Antioxidant activity, Antibacterial activity, *Escherichia coli*, *Salmonella typhi*.

INTRODUCTION:

Medicinal plants are gifts of nature for humans, there are evidential epics that it's been used from the earlier historic era. Plants have the ability to synthesize a wide variety of chemical compounds that are used to the treatment of various diseases according to the World Health Organization (WHO, 2004).

Cleome rutidosperma (Family-Capparidaceae) is a low growing herb, up to 70 cm tall, found in waste herb, grounds and grassy places with trifoliate leaves and small violet blue flower which turn pink as to West Africa although it has become neutralized in various parts of tropical America as well as South East Asia. Different parts of the plant of Cleome genus are used as stimulant, anticorbutic and antihelminthic, vesicant, rubefacient and carminative (Kiritikar and Basu, 1991). The antiplasmodail, locomotors, analgesic, antimicrobial, diuretic and laxative activities of *C. rutidosperma* were also reported (Bidla*et al.*, 2004). The roots of this plant are also reported to have hypoglycemic effect (Mondal *et al.,* 2009).The antimicrobial, antioxidant and anticancer activities were studied using *Cleome* spp. but very few studies were carried out in *C. rutidosperma*. Hence this study was

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aimed to test the positivity of phytochemicalsand study the antioxidant, antibacterial and anticancer activities of the leaf extracts of *C. rutidosperma*.

MATERIALS AND METHODS:

The plant material l(leaves) was collected from forest of Portblair District of Andaman Islands, India during February 2016 and it was authenticated by the Department of Botany, Jawaharlal Nehru Vidyalaya, India. The fresh aerial parts were washed under running tap water for the removal of adhered dirt, followed by rinsed with distilled water, the leaves of the plant are shade dried and then milled into coarse powder by the mechanical grinder. Coarse powder of *C. rutidosperma* leaves was extracted separately with 70%v/v hexane, ethyl acetate, and methanol using a Rotary evaporator. The extracts obtained were dried under reduced pressure at room temperature for the preparation of extracts. Prepared extracts were further used for determination of phytochemical screening, antioxidant, antibacterial and anti cancer activities.

Phytochemical Screening:

Phytochemical screening of the leaf extracts of *C.rutidosperma* was carried out to identify the phytoconstituents such as alkaloids, flavonoids, tannins, saponins, steroids, protein, polyphenols by following standards phytochemical methods (Harborne, 1973).The preliminary screening of phytochemicals of hexane, ethyl acetate and methanolic extracts of *Cleome rutidosperma* was done as follows.

Dragendroff's test for alkaloids:

Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates that the presence of alkaloids.

Alkaline Reagent test for flavonoids:

1 gm of powdered dried leaves was boiled with 10 ml of distilled water for 5 minutes and boiled extract was filtered using Whatman filter paper. Few drops of 20 % sodium hydroxide solution were added to the 1 ml of cooled filtrate. A color change from yellow to colorless when adding acid showed the presence of flavonoids in the extracts.

Ferric chloride test for tannins:

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added in the extract and observed for development of brownish green or a blue-black color..

Foam test for saponins:

Small amount of extract was shaken with little quantity of water. If foam produced persists for ten minutes it indicates the presence of Saponins. For demonstration of frothing**,** 2.5 ml of extract was diluted to 10ml using distilled water and shaken vigorously for 2min. The formation of frothing indicated that the presence of saponinsin the filtrate. For the demonstration of emulsifying properties, 2 drops of olive oil was added to the solution obtained from diluting 2.5 ml filtrate to 10 ml of distilled water and shaken vigorously for a few minutes. The formation of a fairly stable emulsion indicatesthat the presence of saponins.

Libermann Burchard's test for steroids:

Methanol extract was treated with chloroform and filtered using Whatman filter paper No.2. The filtrate was further treated with few drops of acetic anhydride followed by boiling and cooling to room temperature. Finally the concentrated sulphuric acid was added to the mixture and then the formation of brown ring at the junction indicates that the presence of phytosterols.

Polyphenols test for protein:

0.5 ml of a 0.1% solution of ninhydrin is boiled for two minutes with a few ml of extract, a blue color develops indicates presence of protein.1ml of filtered extracts were taken and 1ml of FeCl3 (1%) solution and 1ml of K3(Fe(CN)6) (1%) were added. The appearance of deep blue color indicates the presence of polyphenols.

Antibacterial activity:

Antibacterial activity of the extracts was determined using antimicrobial susceptibility test as described by Bauer *et al*., 1966. The lawn culture of the test organisms was made on the surface of Mueller Hinton Agar (MHA) plate. The bacterial turbidity was compared with McFarland standard 0.5 scale. Wells to add extracts were cut on the agar plate and 50, 100 and 150µg/ml of the plant extracts namely methanol, ethyl acetate and hexane were added in the respective wells against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*. DMSO served as negative control and Streptomycin (30 µg) was used as reference control. Plates were incubated at 37°C for 24 h and the zone of inhibition was noted**.**

Antioxidant activity:

The free radical scavenging capacity of the extract was determined using DPPH 1, 1-diphenyl-2-picrylhydrazyl (DPPH) Assay (Blois, 1958). The antioxidant activities of the extracts were measured by scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) Free radical. 0.1Mm solution of DPPH in methanol (0.2ml) at different concentrations (0.5-500 µg). The mixture was vortexes and allowed to stand in a dark room at room temperature for 30 min. A DPPH blank was prepared without compound and methanol was used for the baseline correction. Ascorbic acid was used as a references standard. Decrease in the absorbance at

517nm was measured using UV-Visible spectrophotometer and the remaining DPPH was calculated. The radical scavenging activity was expressed as the percentage inhibition and was calculated using the following formula: Percentage of Inhibition = [(Ao - A1)/Ao] X 100. Where Ao is the absorbance of the control (without compound) and A1 is the absorbance of the compound. The IC_{50} (concentration causing 50% inhibition) values of each compound was determined graphically.

***In vitro* anticancer activity:**

The HepG2 liver cancer cell line was procured from National Centre for Cell Sciences (NCCS), Pune and used for *in vitro* anticancer study. The *in vitro* cytotoxicity was determined using MTT (3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide) method (Mosmann,1983). The cells were cultured in 96-well cell culture plate. Confluent cells were treated by increasing concentration of methanol extract of C. *rutidosperma* in triplicate wells. The final volume of cell culture medium and methanol extract of *C. rutidosperma* in each well was 90µl. Treated cells were incubated for 24h at 37°C. After 24h, 15µl of MTT solution was added to each well. The microtitre plate was kept at 37°C for an additional 4 hrs in a humidified 5% CO_2atmosphere. After the incubation period, 100µl of the solubilization /stop solution was added to each well of microtitre plate. The 570nm was used to measure the absorbance value of each well. The percentage of cell viability was calculated using the formula like Percentage of Cell Viability = A570 of treated cells / A570 of control cells × 100%.

RESULTS:

The phytochemical screening study reveals that the presence of various groups of phytochemicals like alkaloids, flavonoids, tannins, terpenoids, saponins, polyphenols and proteins in the hexane, ethyl acetate and methanol extracts of *C. rutidosperma*. Among all the three solvent extracts studied, the methanol showed positive for high number of phytochemical groups like alkaloids, flavonoids, tannins, steroids, proteins, polyphenols and terpenoids(Table 1).

Table 1: The positivity of phytochemicals in hexane, ethyl acetate and methanol extracts of *Cleome rutidosperma*

Compounds	Alkaloids	Flavonoids	Tannins	Saponins	Steroids	Protein	Polyphenols	Terpenoids
Hexane Extract	-	-	-	-	+	-	-	-
Ethyl acetate extract	-	++	++	-	++	+++	+++	++
Methanol extract	+++	+++	+++	-	+++	+	+++	+++

+++= Strongly Present; ++= Present; += Traces; -=Not Detected

Antibacterial Activity:

The antibacterial activity of the extracts was tested in the concentration of 50, 100 and 150µg/ml using agar well diffusion method. The hexane extract of leaves of *C. rutidosperma* exhibited the zone of inhibition of 7,8 and 9mm against *Staphylococcus aureus*, 7,8 and 11mm against *Bacillus cereus*, 0,8 and 10mm against *Salmonella typhi* and 0,0 and 8mm against *Escherichia coli*. The ethyl acetate extract exhibited 0,8 and 10mm against *S. aureus*, 8,10 and 10mm against *B. cereus*, 9,11 and 12mm against *S.typhi* and 7,9 and 11mm against *E.coli*. The methanol extract exhibited 0, 0 and 7mm against S. aureus, 7,9 and 12 against *B. cereus*, 8,8 and 10 against *S.typhi* and 9,9 and 12 against *E.coli*. In this study, streptomycin was used as a control and which exhibited the zone of inhibition of 14mm against *S.aureus*, 14 against *B.cereus*, 15 against *S.typhi* and 16mm against *E.coli*(Table 2).

Table 2: Antibacterial activity of leaf extracts of *Cleome rutidosperma*

Name of the Organism	Zone of Inhibition in mm									Positive Control (Streptomycin)	Negative Control (DMSO)
	Hexane Extract (µg)			Ethyl Acetate Extract (µg)			Methanol Extract (µg)				
	50	100	150	50	100	150	50	100	150		
Staphylococcus aureus	7	8	9	0	8	10	0	0	7	14	0
Bacillus cereus	7	8	11	8	10	10	7	9	12	14	0
Salmonella typhi	0	8	10	9	11	12	8	8	10	15	0
Escherichia coli	0	0	8	7	9	11	9	9	12	16	0

DPPH scavenging assay of *C. rutidosperma:*

The antioxidant activity was estimated by DPPH free radical scavenging method and it was expressed in percentage of free radicals scavenging activity. Among three different solvent extracts, methanol extract showed higher percentage of scavenging activity (517.38) followed by ethyl acetate (737.59), hexane (2697.11) and ascorbic acid (5.45). These results reveal that the methanol extract of *C. rutidosperma* leaves having the ability to quench the DPPH radical which indicates that this extract has antioxidant properties with radical scavenging activity with IC(50) of 517.38 (Fig.1).

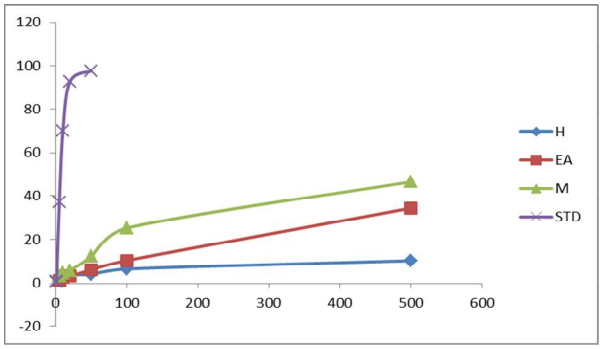
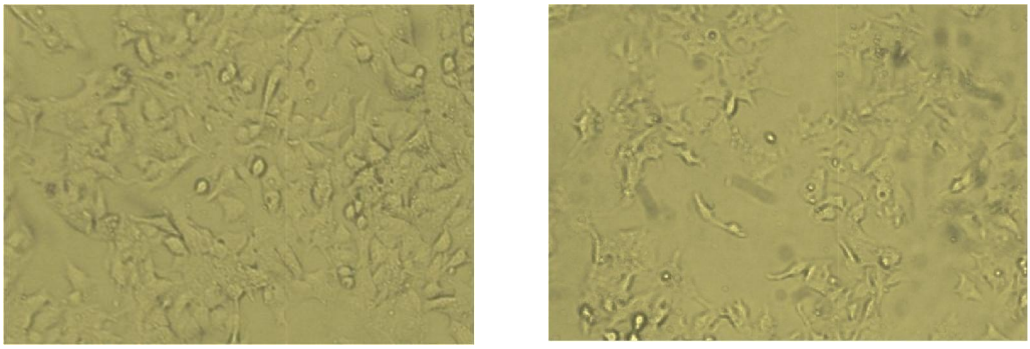


Fig. 1: Graph showing anti-oxidant activity by DPPH method

Anticancer Activity:

Screening of methanolic extract of *C. rutidosperma* results in moderate anticancer activity against HepG2. The maximum concentration (µg/ml) used in the study was 120µg/ml. The cytotoxicity of the methanolic extract was evaluated *in vitro* against human liver cancer cell line (HepG2) at different concentrations like 5µg/ml, 25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml. The cytotoxicity analysis of the samples shown a direct dose-response relationship; cytotoxicity increased at higher concentrations .The methanol extract reveals the considerable cytotoxicity against the HepG2 cancer cell lines. The concentration of methanol extract of from 5 to100 µg/mL shows increase in cytotoxity effect and finally the IC_{50} value was recorded at the concentration of 50 mg/mL for the HepG2cell lines. The methanol leaf extract showed the potent anti-cancer activity on HepG2 cancer cell line and which was confirmed using MTT assay (Fig. 2 and 3).



A HepG2 liver cell lines for control **B HepG2 liver cell lines treated with methanol extract**

Fig.2: HepG2 liver cell lines showing anti cancer activity of methanol extract of leaves of *C. rutidosperma*

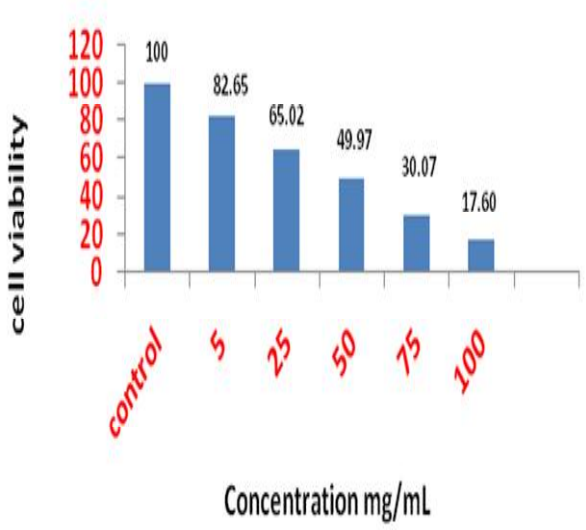


Fig.3: Cell viability of HepG2 cell treated with methanol extract of leaves of *C. rutidosperma*

DISCUSSION:

Nowadays, the pharmaceutical industries were concentrating in the medicinal plants as a source of lead bioactive agents to produce novel drugs. About 25-50% of the modern drugs were derived from medicinal plants. Many medicinal plants were unique in their biological activities but it has been used by different tribes or countries for different ailments, this shows that plants possess a very wide range of healing powers which are attributed to their chemical composition.

Kiritikar and Basu (1991) reported that according to traditional uses, the plant parts like leaves, roots and seeds of *Cleome* genus are used as stimulant, antiscorbutic, anthelmintic, rubifacient, vesicant and carminative. In this study, we used leaves of *C. rutidosperma* to check their antibacterial, cytotoxicity and antioxidant properties and we found that the methanol extract of leaves of *C. rutidosperma* showed significant antibacterial activity against *E.coli* followed by *B.cereus, S. typhi* and *S. aureus*. The study of Bose *et al.* (2007a) reveals that the whole plant extracts using

ethanol, petroleum ether, ethyl acetate and n-butanol showed antibacterial activity against *S.aureus, Streptococcus faecalis, Bacillus polymyxa, B. subtilis, Pseudomonas aeruginosa, Vibrio cholerae, Salmonella typhi* and *Shigella flexineri.* In another study, Bose *et al.* (2007b) reported that the aqueous extract of *C. rutidosperma* exhibited significant antibacterial activity against the both Gram-negative and Gram-positive bacteria. Venkatadri *et al.* (2016) reported that the phytochemical compounds like alkaloids, flavanoids, tannins and phenolic compounds in the medicinal plants were responsible for effective antimicrobial property.

Bose *et al.* (2007a) reported that the phytochemicals namely lipids, steroids, terpenoids, flavonoids, tannins, saponins and sugars in the methanol extract of whole plant of *C.rutidosperma* and ethyl acetate extract of this plant exhibited positivity to the presence of phytochemical constitutents like flavonoids, tannins and triterpenoids. The positivity of phytochompounds present in the *C.rutidosperma* were correlated with our study that the methanol extract of leaves of *C.rutidosperma* exhibited positivity to presence of alkaloids, flavonoids, tannins, steroids, proteins, polyphenols and terpenoids and ethyl acetate extract exhibited positivity to the presence of phytochemical constituents like flavonoids, tannins and triterpenoids.

Bose *et al.* in 2008 studied the antioxidant and free radical scavenging activity of *C. rutidosperma.* They reported that this plant has potential source of natural antioxidant and they suggested that the flavonoids, tannins and terpenoids present in this plant may be responsible for the antioxidant activity. The isolation and identification of antioxidant components from this plant may useful in the clinical settings. In this study, we found that the methanol extract of leaves of *C. rutidosperma* showed highest scavenging activity.wed by ethyl acetate and hexane. In addition with antibacterial and antioxidant activities, this study also reports that the anticancer activity of leaves extract of *C. rutidosperma*. In this current study, the methanol extract showed considerable cytotoxicity against HepG2 cancer cell line.

CONCLUSION:

To the best of our knowledge, this study reports first time the antioxidant activity of *Cleome rutidosperma* and it is concluded that the plant *Cleome rutidosperma* possesses many phytochemicals and the leaf extracts showed significant antioxidant, antibacterial and cytotoxicity properties.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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