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P Kanimathi
PG and Research Department of Botany, Bharathi Women's College (A), Chennai, Tamil Nadu, India

KS Ramya
PG and Research Department of Botany, Bharathi Women's College (A), Chennai, Tamil Nadu, India

Dr. A Radha
PG and Research Department of Botany, Bharathi Women's College (A), Chennai, Tamil Nadu, India

Corresponding Author:
P Kanimathi
PG and Research Department of Botany, Bharathi Women's College (A), Chennai, Tamil Nadu, India

GC-MS analysis and antibacterial activity of *Cleome rutidosperma, Cleome gynandra* and *Cleome viscosa* seed extracts: A comparative study

P Kanimathi, KS Ramya and Dr. A Radha

Abstract
Phytocomponents present in the chloroform seed extracts of *Cleome rutidosperma, Cleome gynandra* and *Cleome viscosa* species were analayzed by Gas Chromatography – Mass Spectrometry method. The air dried seeds were powdered and subjected to chloroform and methanol solvent extraction. Then each of these extracts was further subjected to Gas Chromatography-Mass spectrometry and its antibacterial activity analyzed. Qualitative determination of the different biologically active compounds from the crude extracts of *Cleome* species seeds using Gas Chromatography-Mass Spectrometry revealed different types of high and low molecular weight chemical entities with varying quantities present in each of the extracts. These chemical compounds are considered biologically and pharmacologically important. The extracts were found to possess significant dose dependent antibacterial activity against both Gram positive and Gram negative bacteria. This study confirmed antibacterial property of the plants studied and its potential as a source of plant based drug.

Keywords: *Cleome rutidosperma* DC, *Cleome gynandra* L, *Cleome viscosa* L, GC-MS analysis, antibacterial activity

1. Introduction
In India, more than 3,500 plant species are used in the preparation of natural drugs [1]. According to World Health Organization for primary healthcare needs more than 80% of World's population depend on traditional medicine [2]. In different countries plants are used as a source of many potent and powerful drugs [3,4]. Due to less availability and high cost of new generation antibiotics alternative medicines with claimed antimicrobial activity is the need of the hour [5].

Cleome genus, with nearly 200 species of annual or perennial herbaceous plants, is the largest genus from *Cleomeaceae* family. According to folk information *Cleome rutidosperma* DC., an annual herb native to West Africa, is used by tribe people [6, 7]. The plant roots exhibit hypoglycemic, anthelmintic activity, while the aerial parts have diuretic, antimicrobial, anti-inflammatory, antioxidant and wound healing properties [8-13].

Cleome gynandra L is a weed, native to Africa [14]. Leaves have anti-inflammatory, disinfectant and anti-tick properties. Stem exhibits antinociceptive and anti-inflammatory activity. Leaf juice and oil are used for ear ache and eye wash. Seeds with antihelmintic properties and seed oil used as fish poison are also reported [15, 16].

Cleome viscosa L native to Asia, is a small herb found in grassy places [17]. The plant is reported to have antimalarial activity, used for uterine complaints, leprosy, blood disease, fevers and wound healing. The seeds exhibit anthelmintic, detergent, antidiarrheal and fever reducing properties. Fresh juice of the seeds is used for mental disorders and infantile convulsions [18-21].

This study has been undertaken to investigate the presence of bioactive compounds through GC-MS analysis and the antibacterial activity of *C. rutidosperma*, *C. viscosa*, and *C. gynandra* seed extracts, as there are no published reports so far with the above objective.

2. Materials and methods
2.1 Plant sample and crude extract preparation
The plant samples were collected from Chennai, Tamil Nadu, identified and the herbarium is preserved in our research laboratory for future reference. The voucher specimen number is 4037, 4038 and 4036 for *C. rutidosperma*, *C. gynandra* and *C. viscosa* respectively. The seeds were collected and dried under shade. After drying, it was powdered and subjected to chloroform and methanol solvent extraction. The extract was then dried at room temperature.

2.2 GC-MS analysis

The GC-MS analysis of bioactive compounds from different extracts of the seeds of the selected plants was done at "VIT-SIF Lab, SAS, Chemistry Division for NMR and GC-MS Analysis. The Clarus 680 GC used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The extract sample (1µl) was injected into the instrument and the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min^{-1}; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The scanned fragments ranged from 40 to 600Da. Relative quantities of the chemical compounds present in each of the seed extracts were expressed as percentage based on peak area produced in the chromatogram.

2.3 Identification of chemical constituents

Bioactive compounds detected in the different extracts of *C. rutidosperma*, *C. gynandra* and *C. viscosa* seeds were identified based on GC retention time on Elite 5MS column and the spectrum of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

2.4 *In vitro* antibacterial activity

To evaluate the antibacterial activity of the selected plant seed extracts ten different strains of bacteria were tested which were obtained from TNAU, Coimbatore; three Gram positive bacteria namely *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus* and seven Gram negative bacteria namely *Salmonella typhi*, *Salmonella paratyphi*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia* and *Enterobacetrium* species.

The antibacterial activity of different seed extracts was determined by agar well diffusion method [22]. Sterilized nutrient agar (Hi-media) medium was poured in sterile Petriplates and bacterial strains were spread on it. The extract was dissolved in DMSO (10mg/ml) and wells were filled with 50, 100, 150, 200 and 250µl. Ampicillin dissolved in distilled water (1mg/ml) was used as positive control and DMSO as negative control. Plates were incubated at 37°C for 24 h and zone of inhibition was measured in mm.

2.5 Statistical analysis

All the experiments were carried out in triplicates. The results are expressed as mean ± standard errors and the comparison of the antibacterial activity of the samples with standard antibiotic was evaluated by applying one way analysis of variants.

3. Results

3.1 Percentage yield and physical properties

From the three batches of approximately 500g of air dried seeds, the yield obtained and the physical properties of the extracts is presented in Table 1. Among the six extracts chloroform extract of *C. viscosa* has the highest percentage yield.

Table 1: Percentage yield and physical properties of extracts

Plant Name	Solvent	Color	Odor	% yield(g)
C. rutidosperma	Chloroform	Light brown	Sour unpleasant odor	3.95
	Methanol	Reddish black	Pungent smell	1.27
C. gynandra	Chloroform	Dark green	Citrus odor	0.6
	Methanol	Dark green	Strong citrus odor	2.46
C. viscosa	Chloroform	Brown	Agreeable odor	27.5
	Methanol	Orange	Strong agreeable odor	1.99

3.2 Bioactive compounds present in the extracts

The bioactive compounds present in chloroform and methanol seed extracts of *C. rutidosperma*, *C. gynandra* and *C. viscosa* are shown in Table 2-4. Their identification and characterization were based on their elution order in HP-5MS column. The compound name, molecular formula, retention time and area % of these bioactive compounds are also presented. Major components present in *C. rutidosperma* Chloroform Extract (CRCE), *C. rutidosperma* Methanol Extract (CRME), *C. gynandra* Chloroform Extract (CGCE), *C. gynandra* Methanol Extract (CGME), *C. viscosa* Chloroform Extract (CVCE) and *C. viscosa* Methanol Extract (CVME) are E-2-Octadecadecen-1-ol (95.593%), Cis-9,10-Epoxyoctadecan-1-ol (71.727%), Pentanoic acid, 10-undecenyl ester (67.456%), Ethyl oleate (31.078%), 9,12-Octadecadienoyl chloride (Z,Z)- (72.668%), 9,12-Octadecadienoic acid (Z,Z)- (86.526%) respectively (Figure 1). The GC-Chromatograms of the six extracts presented in Figure 2-7 shows the retention time in the column and detected peaks which correspond to the bioactive compounds present in chloroform and methanol seed extracts.

Table 2: Biologically active chemical compounds of CRCE and CRME

Sample	Name of compounds	Molecular Formula	Retention time (min)	Area %
CRCE	N-Hexadecanoic acid	$C_{16}H_{32}O_2$	19.285	04.407
	E-2-Octadecadecen-1-ol	$C_{18}H_{36}O$	20.375	95.593
CRME	Heptacosanoic acid, Methyl ester	$C_{28}H_{56}O_2$	17.774	01.580
	Heneicosanoic acid, methyl ester	$C_{22}H_{44}O_2$	18.860	01.693
	Oleic acid	$C_{18}H_{34}O_2$	20.105	09.578
	Pentadecanoic acid, 2,6,10,14-Teramethyl-, Methyl ester	$C_{20}H_{40}O_2$	20.240	04.566
	Pentadecanoic acid	$C_{15}H_{30}O_2$	20.440	05.672
	Cis-9,10-Epoxyoctadecan-1-ol	$C_{18}H_{36}O_2$	21.391	71.727
	11-Tridecen-1-ol	$C_{13}H_{26}O$	24.197	03.574
	Pseduosarasasapogenin-5,20-Dien methyl ether	$C_{28}H_{44}O_2$	27.658	01.609

Table 3: Biologically active chemical compounds of CGCE and CGME

Sample	Name of compounds	Molecular Formula	Retention time (min)	Area %
CGCE	N-Hexadecanoic acid	$C_{16}H_{32}O_2$	18.955	11.389
	Pentadecanoic acid	$CH_3(CH_2)_{13}COOH$	19.205	06.687
	N-Hexadecanoic acid	$C_{16}H_{32}O_2$	19.440	02.287
	6,10-Dimethyl-4-undecanol	$C_{13}H_{28}O$	19.680	02.574
	Pentanoic acid, 10-undecenyl ester	$C_{16}H_{30}O_2$	20.225	67.456
	1,19-Eicosadiene	$C_{20}H_{38}$	21.171	09.607
CGME	Hexadexanoic acid, ethyl ester	$C_{18}H_{36}O_2$	18.245	28.424
	Ethyl oleate	$C_{20}H_{38}O_2$	19.550	31.078
	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	19.755	19.018
	N-Hexadecanoic acid	$C_{16}H_{32}O_2$	20.451	08.996
	Oleic acid	$C_{18}H_{34}O_2$	20.576	07.368
	1-Octadecyne	$C_{18}H_{31}$	20.886	02.538
	9-Methyl-Z,Z-10,12,Hexadecadien-1-ol acetate	$C_{19}H_{34}O_2$	21.721	02.577

Table 4: Biologically active chemical compounds of CVCE and CVME

Sample	Name of compounds	Molecular Formula	Retention time (min)	Area %
CVCE	N-Hexadecanoic acid	$C_{16}H_{32}O_2$	18.775	06.573
	11,14-Eicosadienoic acid, Methyl ester	$C_{21}H_{38}O_2$	19.220	15.114
	Octadecanoic acid, Methyl ester	$C_{19}H_{38}O_2$	19.410	03.858
	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}OCl$	19.990	72.668
	Docosanoic acid	$C_{22}H_{44}O_2$	29.724	01.787
CVME	N-Hexadecanoic acid	$C_{16}H_{32}O_2$	19.265	05.798
	1-Tetradecyne	$C_{14}H_{26}$	19.565	04.209
	11-Hexadecynal	$C_{16}H_{28}O$	20.000	03.467
	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	20.491	86.526

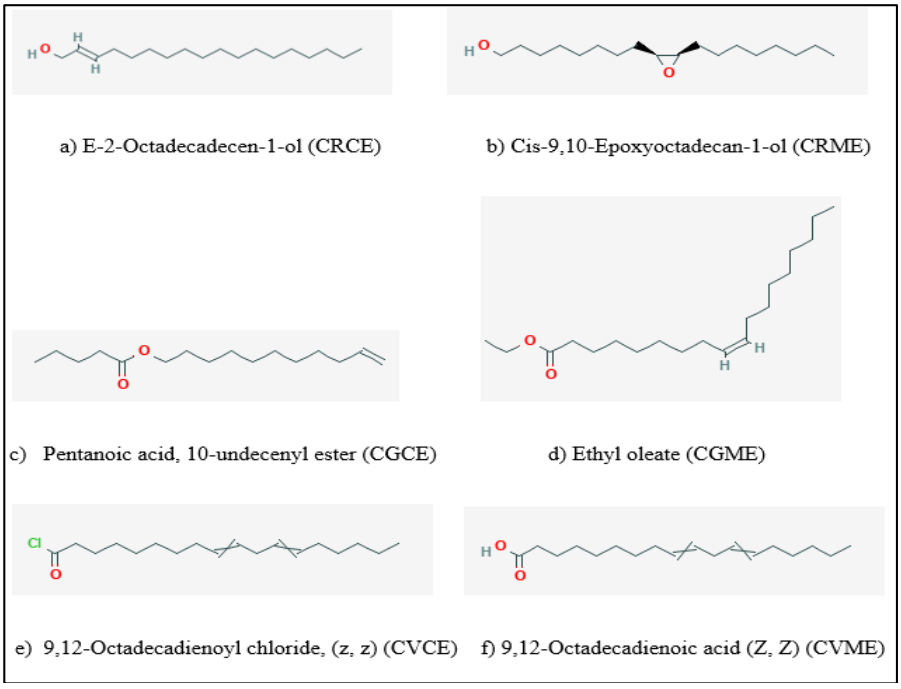

Fig 1: Major components present in the seed extracts

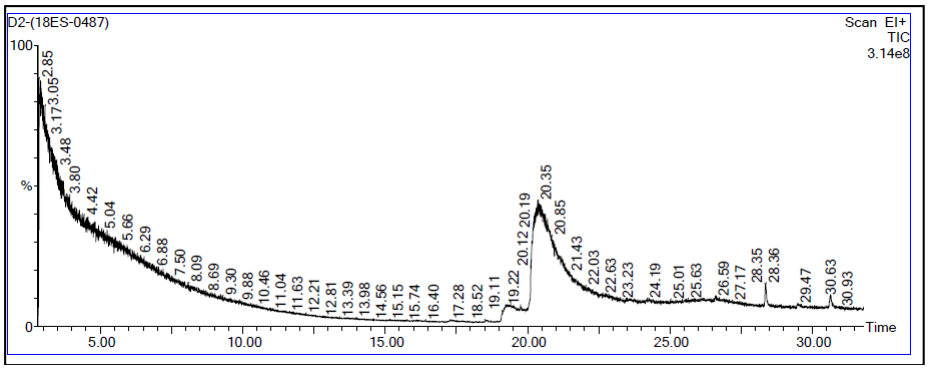

Fig 1: A typical chromatogram of the bioactive compounds present in CRCE

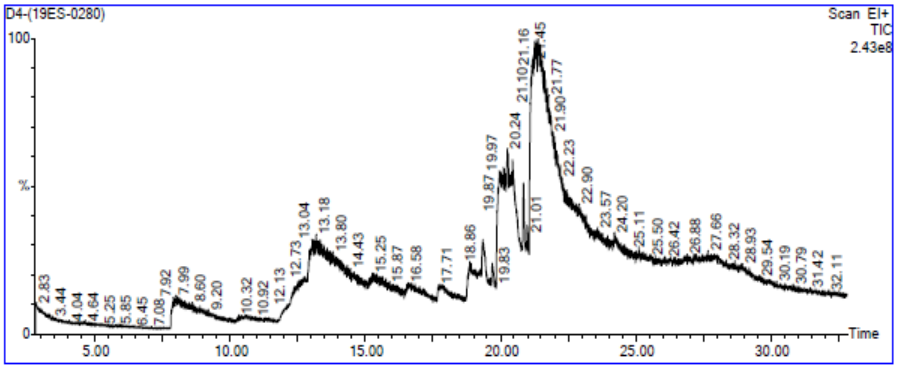

Fig 2: A typical chromatogram of the bioactive compounds present in CRME

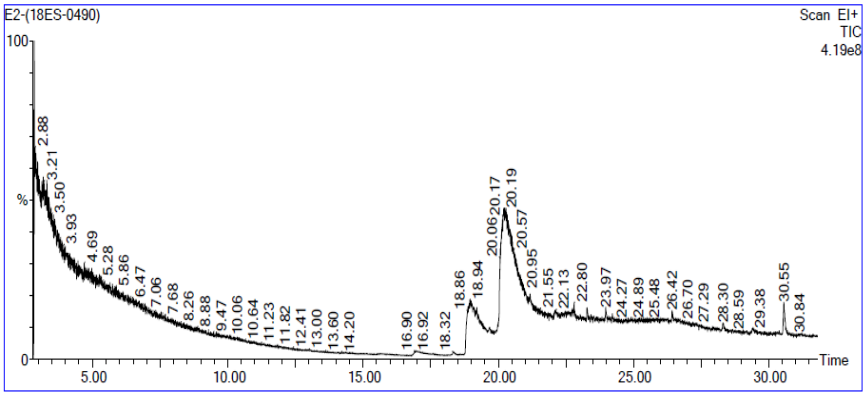

Fig 3: A typical chromatogram of the bioactive compounds present in CGCE

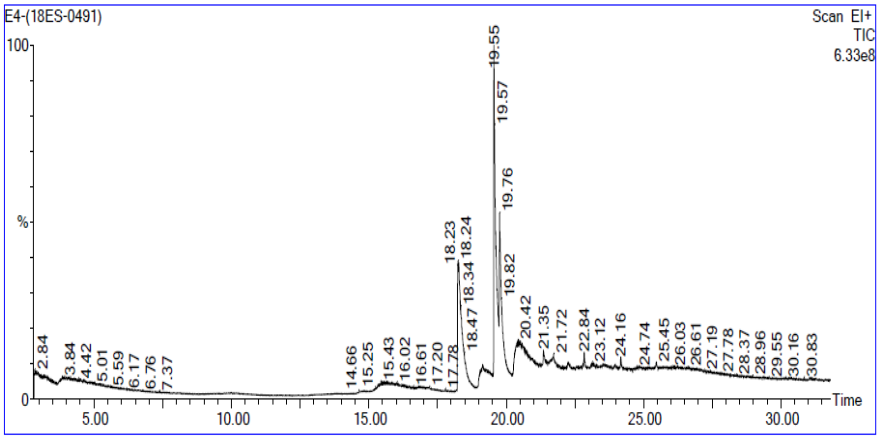

Fig 4: A typical chromatogram of the bioactive compounds present in CGME

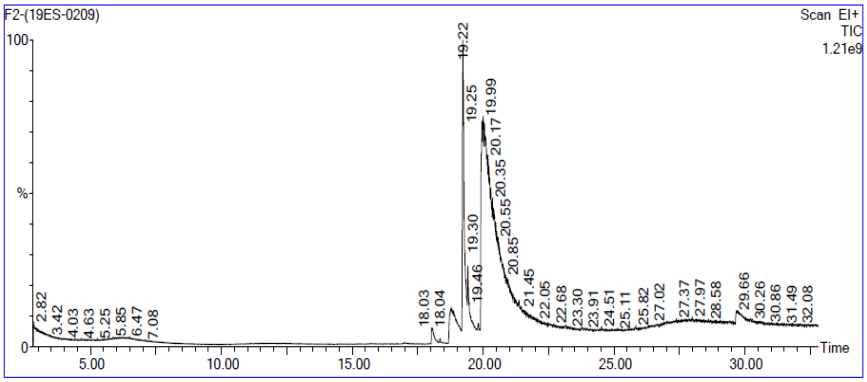

Fig 5: A typical chromatogram of the bioactive compounds present in CVCE

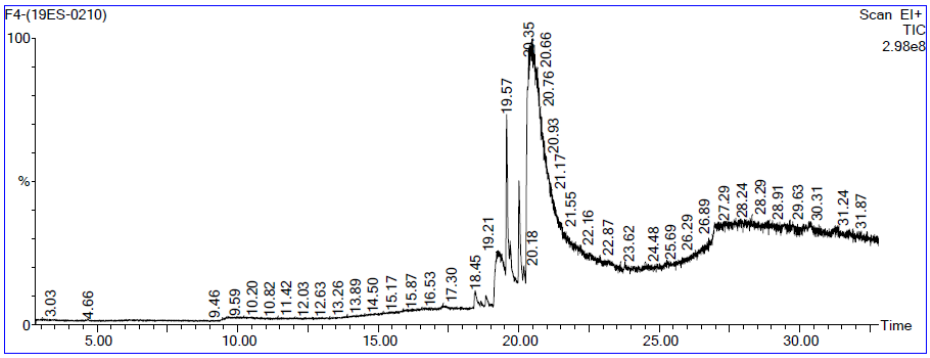

Fig 6: A typical chromatogram of the bioactive compounds present in CVME

3.3 Antibacterial activity

The various extracts of *Cleome* species showed significant antibacterial activity. The results of antibacterial activity are presented in Table 5-7 and Figure 8-13. Inhibitory activity was prominent from 150 µl concentration onwards.

Table 5: Antibacterial activity of CRCE and CRME

Name of pathogen	Sample	Ampicillin (5µl)	Dmso	50µl	100µl	150µl	200µl	250µl
B. cereus	CRCE	14 ± 0.09	-	-	-	12 ± 0.04	14 ± 0.09	15 ± 0.08
	CRME	22 ± 0.09	-	-	-	12 ± 0.08	14 ± 0.04	16 ± 0.09
B. subtilis	CRCE	14 ± 0.09	-	-	-	-	-	-
	CRME	22 ± 0.09	-	-	-	-	-	-
S. aureus	CRCE	14 ±0.09	-	-	-	-	-	-
	CRME	22 ± 0.09	-	-	-	-	-	-
S. typhi	CRCE	15 ± 0.04	-	-	12 ± 0.04	14 ± 0.04	16 ± 0.04	17 ± 0.42
	CRME	15 ± 0.04	-	-	-	-	-	-
S. paratyphi	CRCE	15 ± 0.04	-	-	-	-	-	-
	CRME	22 ± 0.08	-	-	12 ± 0.04	14 ± 0.04	16 ± 0.08	17 ± 0.04
P. vulgaris	CRCE	14 ±0.09	-	-	-	-	15 ± 0.04	17 ± 0.04
	CRME	20 ± 0.04	-	-	-	13 ± 0.09	16 ± 0.08	18 ± 0.04
P. aeruginosa	CRCE	15 ±0.04	-	-	-	-	-	-
	CRME	22 ± 0.09	-	-	-	-	-	-
E. coli	CRCE	14 ±0.08	-	-	-	14 ± 0.04	16 ± 0.09	17 ± 0.04
	CRME	20 ± 0.04	-	-	13 ± 0.08	15 ± 0.04	17 ± 0.09	19 ± 0.03
K. pneumoniaea	CRCE	14 ±0.08	-	-	-	-	-	-
	CRME	22 ± 0.09	-	-	-	-	-	-
Entero bacterium sp.	CRCE	14 ±0.08	-	-	-	-	-	-
	CRME	20 ± 0.04	-	-	-	-	-	-

Data given are mean of triplicates. ± standard error

Table 6: Antibacterial activity of CGCE AND CGME

Name of pathogen	Sample	Ampicillin (5µl)	Dmso	50µl	100µl	150µl	200µl	250µl
B.cereus	CGCE	14 ± 0.09	-	-	-	15 ± 0.09	17 ± 0.08	18 ± 0.04
	CGME	16 ± 0.09	-	-	-	11 ± 0.09	13 ± 0.04	13 ± 0.04
B.subtilis	CGCE	14 ± 0.09	-	-	-	-	-	-
	CGME	20 ± 0.08	-	-	13 ± 0.12	16 ± 0.04	17 ± 0.04	19 ± 0.08
S.aureus	CGCE	14 ±0.04	-	-	-	-	11 ± 0.03	12 ± 0.04
	CGME	16 ± 0.08	-	-	-	10 ± 0.23	12 ± 0.04	15 ± 0.04
S.typhi	CGCE	14 ± 0.09	-	-	-	-	-	-
	CGME	18 ± 0.04	-	-	-	12 ± 0.08	14 ± 0.04	15 ± 0.04
S.paratyphi	CGCE	14 ± 0.09	-	-	-	-	-	-
	CGME	16 ± 0.04	-	-	-	-	10 ± 0.04	12 ± 0.04
P.vulgaris	CGCE	15 ±0.04	-	-	-	15 ± 0.04	16 ± 0.08	17 ± 0.09
	CGME	18 ± 0.04	-	-	-	11 ± 0.04	14 ± 0.04	15 ± 0.04
P.aeruginosa	CGCE	15 ±0.04	-	-	-	-	-	-
	CGME	16 ± 0.08	-	-	-	-	-	-
E.coli	CGCE	14 ±0.08	-	-	-	16 ± 0.03	17 ± 0.04	18 ± 0.08
	CGME	16 ± 0.04	-	-	-	-	-	-
K.pneumoniaea	CGCE	15 ±0.04	-	-	-	-	-	-
	CGME	18 ± 0.04	-	-	-	-	-	-
Enterobacteriumsp.	CGCE	15 ±0.04	-	-	-	-	-	-
	CGME	18 ± 0.04	-	-	-	-	-	-

Data given are mean of triplicates. ± standard error

Table 7: Antibacterial activity of CVCE and CVME

Name of pathogen	Sample	Ampicillin (5µl)	Dmso	50µl	100µl	150µl	200µl	250µl
B.cereus	CVCE	14 ± 0.04	-	-	-	16 ± 0.03	17 ± 0.08	18 ± 0.04
	CVME	15 ± 0.04	-	-	-	13 ± 0.04	15 ± 0.09	16 ± 0.04
B.subtilis	CVCE	14 ± 0.04	-	-	-	-	-	-
	CVME	16 ± 0.04	-	-	10 ± 0.04	12 ± 0.04	16 ± 0.09	18 ± 0.04
S.aureus	CVCE	14 ± 0.04	-	-	-	-	-	-
	CVME	16 ± 0.04	-	-	12 ± 0.04	15 ± 0.04	18 ± 0.09	20 ± 0.04
S.typhi	CVCE	14 ± 0.04	-	-	-		-	-
	CVME	15 ± 0.04	-	-	-	-	-	-
S.paratyphi	CVCE	14 ± 0.04	-	-	-	-	-	-
	CVME	17 ± 0.04	-	-	-	13 ± 0.08	14 ± 0.09	15 ± 0.04
P.vulgaris	CVCE	13 ± 0.09	-	-	-	-	10 ± 0.04	11 ± 0.08
	CVME	18 ± 0.04	-	-	-	-	-	-
P.aeruginosa	CVCE	13 ± 0.09	-	-	-	-	-	-
	CVME	18 ± 0.04	-	-	-	12 ± 0.04	14 ± 0.04	15 ± 0.04
E.coli	CVCE	14 ± 0.08	-	-	-	15 ± 0.04	16 ± 0.09	17 ± 0.05
	CVME	18 ± 0.04	-	-	11 ± 0.09	15 ± 0.04	19 ± 0.09	23 ± 0.09
K.pneumoniaea	CVCE	13 ± 0.09	-	-	-	-	-	-
	CVME	17 ± 0.04	-	-	-	-	-	-
Enterobacterium sp.	CVCE	13 ± 0.09	-	-	-	-	-	-
	CVME	18 ± 0.04	-	-	-	-	-	-

Data given are mean of triplicates. ± standard error

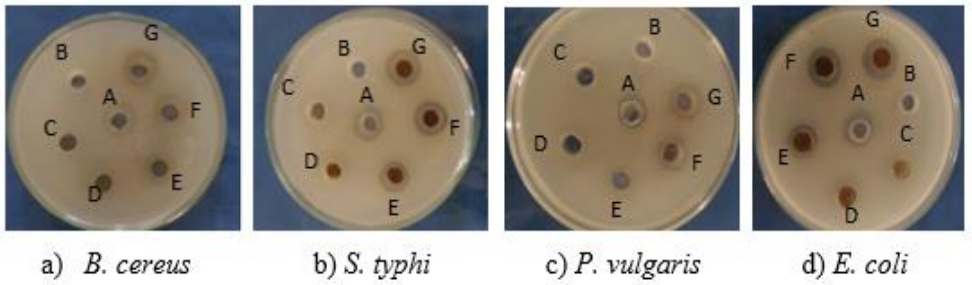

Fig 8: Antibacterial activity of CRCE

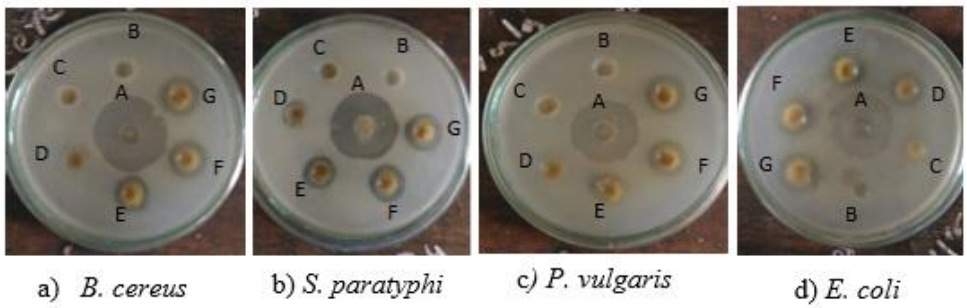

Fig 9: Antibacterial activity of CRME

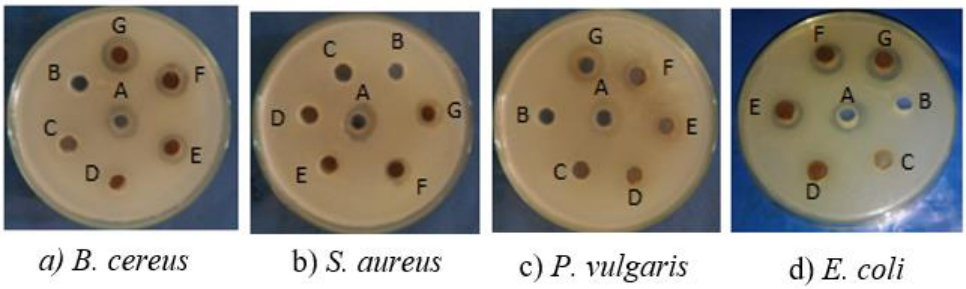

Fig 10: Antibacterial activity of CGCE

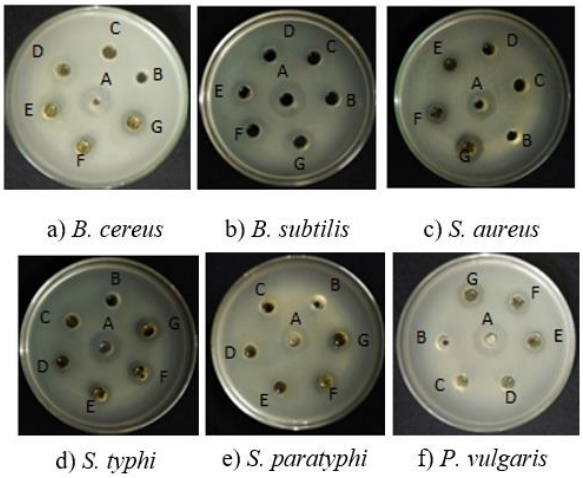

Fig 11: Antibacterial activity of CGME

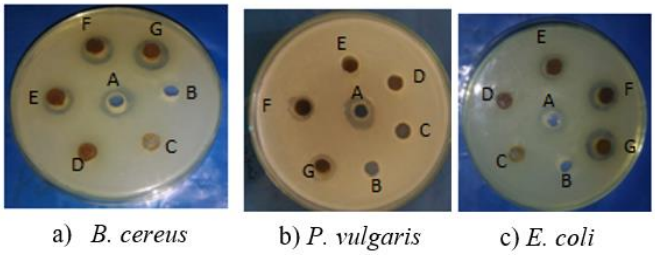

Fig 12: Antibacterial activity of CVCE

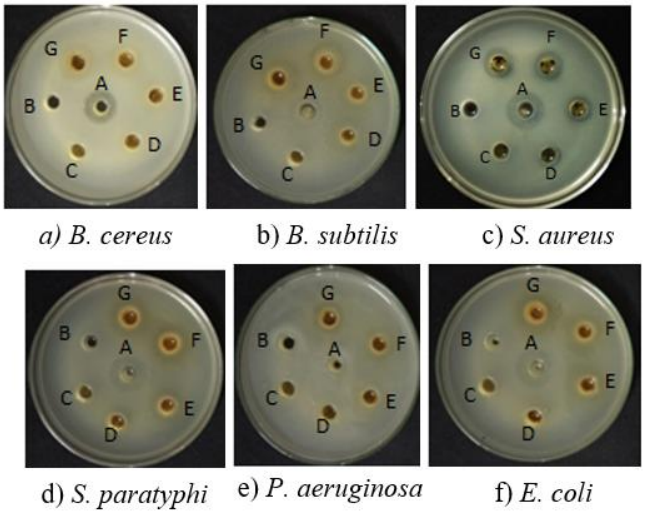

Fig 13: Antibacterial activity of CVME [A-Positive control (Ampicillin), B-Negative control (DMSO), C-50 µl, D-100 µl, E-150 µl, F-200 µl and G-250 µl]

4. Discussion

In the present study, no common major compounds were present in CRCE, CRME, CGCE, CGME, CVCE and CVME of seeds. But the presence of n-hexadecanoic acid, a minor compound, was detected in the seed extracts of all the plants except in CRCE. Based on literature, some of the constituents revealed by GC-MS are biologically active compounds. The compound n-hexadecanoic acid, exhibits biological activity such as hypocholesteromic, nematicide, lubricant, antimicrobial, diuretic, anticancer, antioxidant, pesticide, antiandrogenic and anti-inflammatory [23]. Ethyl oleate present in CGCE is used to treat hepatic cancer. It is also used as an agent for antimicrobial activity, food flavoring, plateing, surface treating and as lubricants [24]. The compound present in CVCE 9, 12-Octadecadienoyl chloride, (Z,Z) plays an important role in the treatment of diabetic retinopathy [25]. The compound from CVME 9, 12-Octadecadienoic acid, also known as linolenic acid, is used as a best dietary fatty acid for breast and prostate cancer, prevention of pre-eclampsia as an anti-inflammatory, insectifuge, cancer preventive, antihistaminic, antiarthritic, antieczemic, nematicide, hypocholesterolemic, hepatoprotective and antiacne [26]. Pentanoic acid, 10-undecenyl ester, a fatty acid ester, detected in CGCE, is reported to have antioxidant property [27,28].

Previous literature studies of *Cleome burmanni* leaf chloroform extract GC-MS analysis reported the presence of sixteen compounds [29]. Chloroform extracts of whole wild plant and callus of *C. viscosa*, didn't reveal any common compound. The GC-MS analysis of the ethanolic extracts of whole wild plant and callus of *C. viscosa* revealed two common compounds namely Tetradecanoic acid and 4',5,7-Trihydroxy isoflavone. Hexadeconoic acid was the major compound present in *C. gynandra* ethyl acetate and ethanol leaf extract and in *C. viscosa* ethanol extract of callus [30, 31]..

The antibacterial activity of *Cleome* species seed extracts was found to be concentration dependent. The study revealed that the extracts were effective against both Gram positive and Gram negative bacteria. All the six extracts showed prominent antibacterial activity against *B. cereus* when compared to the antibiotic. Antibacterial activity of CRCE, CGCE and CVME was significant when compared to the standard antibiotic against *E. coli, S. typhi, S. paratyphi* and *P. vulgaris.* The growth of *B. subtilis* was effectively inhibited only by CGME and CVME which more or less equal to the standard antibiotic. The growth of *S. aureus* was inhibited by CGCE, CGME and CVME from 100 µl onwards. Only CVME could inhibit the growth of *P. aeruginosa* in a dose dependent manner starting from 150 µl up to 250 µl concentration.

Earlier studies on the above mentioned plant extracts of various parts reveal antibacterial activity. Water, ethyl acetate, 90% ethanol, petroleum ether, and diethyl ether extracts of the whole plant and leaves of *C. rutidosperma* exhibited antibacterial activity. Significant growth inhibitory activity was observed in ethyl acetate extract of leaves [11, 32]. In *C. gynandra* various parts like leaves, roots, stems, seeds and seed pods also show antibacterial activity. Among them only benzene crude extracts of leaves and seeds revealed predominant antibacterial activity. Benzene seed extract of *C. gynandra* inhibited the growth of *Agrobacterium tumifaciens* with a zone size of 28 mm [33]. Ethanol extract of whole plant, leaves and seeds of *C. viscosa* also revealed prominent antibacterial activity against *K. pneumoniaea* where 18 mm inhibition zone was recorded [34, 35].

The identification of biologically active compounds by GC-MS analysis and antimicrobial activity of *C. rutidosperma, C. gynandra* and *C. viscosa* seed extracts support the medicinal uses of these plants. Owing to the growing concern of antimicrobial resistance, further purification and characterization of the compounds, directed by bioactivity-guided assay, will serve the basis in determining the antimicrobial potential of these plants against a wide spectrum of microbes.

5. Conclusion

The present study is the first report on the GC-MS analysis in *Cleome rutidosperma, C. gynandra* and *C. viscosa* seed extracts. Various chemical constituents were identified from the chloroform and methanol extract of the plant. Presence of

medicinally useful phytocomponents in the extract implies the phytopharmaceutical importance of the plant. Further studies are to ascertain the pharmacological activity of the concerned compounds, their isolation and characterization are in progress

Conflict of interest statement

We declare that we have no conflict of interest.

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