



Chemical composition, anthelmintic and antimicrobial activity of the essential oil of the aerial part of *Cleome rutidosperma*

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Abstract

Background and Aim: *Cleome rutidosperma* is extensively employed in herbal medicine. The aim of this study is to determine the chemical constituents, anthelmintic and antimicrobial activity of the essential oil from the aerial part of *Cleome rutidosperma*.

Materials and methods: Fresh aerial part of *Cleome rutidosperma* was ground and steam distilled to get its essential oil. Gas chromatography was used to separate the constituents of the essential oil while the individual constituents were identified by mass spectrometry. Antimicrobial sensitivity test for the essential oil was carried out using agar disc diffusion method while minimum inhibitory, minimum bactericidal and minimum fungicidal concentrations were determined through broth dilution method. 1%, 5% and 10% solutions of the essential oil were used for determination of its anthelmintic activity against *Lumbricus terrestris*.

Results: The essential oil contains forty eight natural products which are being identified for the first time in *Cleome rutidosperma*. The oil exhibited antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Candida albicans* and *Aspergillus niger*.

Conclusion: The present study shows that the essential oil has anthelmintic activity against *Lumbricus terrestris* and antimicrobial activity against the test organisms. It also has anthelmintic activity against earth worm.

Keywords: *Cleome rutidosperma*, *Lumbricus terrestris*, antimicrobial activity, anthelmintic activity, essential oil, chemical constituents

1. Introduction

Cleome rutidosperma is an annual plant and a very common weed in farmlands. Different parts of the plant are employed in phytotherapy for treatment of parasitic skin diseases, earache, malaria, paralysis, epilepsy, convulsion, spasm and pains etc. ^[1]. Aqueous extract of the plant has anthelmintic activity against the cattle parasite, *Haemorchus contortus* ^[2]. Its ethyl acetate extract has antibacterial and bio-enhancing activity against multidrug resistant clinical isolates ^[3]. It also shows marked anti-depressant ^[4] as well as diuretic and antibacterial activity ^[5, 6]. It also has wound healing ^[7], antimicrobial ^[8], and anticonvulsant ^[9], anti-arthritis ^[10], antifungal ^[11] and anti-diabetic ^[12] activity. The leaf has bitter taste like mustard ^[13].

Although *Cleome rutidosperma* has a lot of therapeutic value and has been extensively employed in phytotherapy ^[1-12], there is very scanty report on its chemical composition. To the best of our knowledge, there is no previous report on its essential oil. Many pathogenic microbes have developed resistance to a number of conventional drugs employed in orthodox medical practice. As a result, there is an ongoing revival on the use of herbal drugs for control of infections and this is becoming increasingly popular. These herbal drugs have the added advantage of being readily available, environmentally friendly and cheap ^[14].

Cleome rutidosperma is a versatile African medicinal plant. The present work is therefore aimed at isolation and determination of the chemical constituents of its essential oil

It is also aimed at evaluation of its antimicrobial and anthelmintic activity.

2. Materials and methods

2.1. Isolation of essential oil

Fresh aerial part of *Cleome rutidosperma* was harvested and rinsed with distilled water. The fresh material was crushed and subjected to steam distillation for 3h using Clevenger's apparatus. The essential oil which separated out from the steam distillate was collected with the help of a separating funnel.

2.2 GC-MS analysis of the essential oil

Separation and identification of the components of the essential oil was achieved through GC-MS analysis using Agilent Hewlett Packard (7980A) with triple detector equipped with auto-injector (10µm syringe). Helium was used as the carrier gas. The column length is 30cm, internal diameter 0.25 µm, thickness 250 µm, treated with phenylmethylsiloxane. Ion source temperature is 250°C, pressure 16.2psi, 1 µm injection in split mode with split ratio of 1.50, with injection temperature of 300°C. The column temperature was raised at 35°C for 5min and changed to 150°C at the rate of 20°C min⁻¹ and held for 5 min before ionization. Microsoft solution software provided by the supplier was used to control the system and to acquire the data. Identification of the compounds was carried out by comparing the mass spectra obtained with those of the

standard mass spectra from National Institute of Standard and Technology, NIST.

2.3. Antimicrobial susceptibility test

Agar disc diffusion method was used for determining the antimicrobial susceptibility of gram negative *Escherichia coli* and *Pseudomonas aeruginosa*; gram positive *Staphylococcus aureus* and *Streptococcus faecalis* and the fungi *Candida albicans* and *Aspergillus niger*. All these are clinical isolates. The microbes were cultured and maintained using the method of Crickshank [15]. Solutions of the essential oil containing 100, 50, 25, 12.5 and 6.25 μgcm^{-3} were prepared. Soboarand's agar and nutrient agar were used for fungi and bacteria respectively. Sterilized filter paper disc were separately soaked in the different solution containing different levels of the essential oil. They were placed on different plates containing the different test organisms. They were incubated at 37°C for 24h. After incubation the zone of inhibition was observed and recorded for the different plates. 100mg of doxycycline was dissolved in 1000cm³ of distilled water to give a 100 μgcm^{-3} solution. This was diluted to get a 10 μgcm^{-3} solution of doxycycline which was used as the control.

2.4. Minimum inhibitory concentration

The minimum inhibitory concentration was done using the method of Razafintsakins [16, 17]. Solutions containing 1.57, 3.13, 6.25, 25, 50 and 100 μgcm^{-3} of the essential oil were prepared. 1cm³ of each of the solutions was placed in separate test tubes. 4cm³ of Mueller Hinton broth was added to each of the test tubes and shaken gently. The test tubes were sealed with sterilized corks and incubated for 24h at 37°C. The test

tube with high degree of clearance is taken as the minimum inhibitory concentration and the one preceding it is minimum bactericidal or minimum fungicidal concentration for bacteria and fungi respectively. The procedure was separately carried out for *Escherichia coli*, *Candida albicans*, *Aspergillus niger*, *Streptococcus faecalis* and *Pseudomonas aeruginosa*.

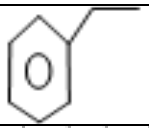

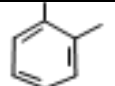
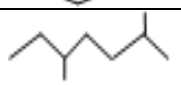
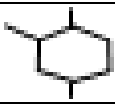
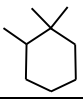
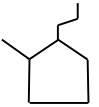
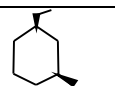
2.5. Anthelmintic test

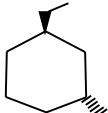
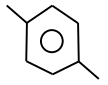


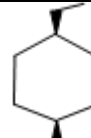
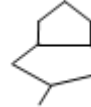
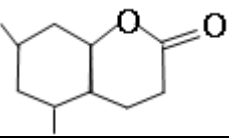
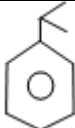
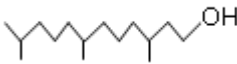
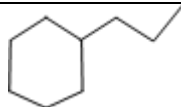
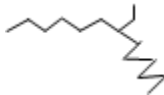

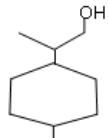
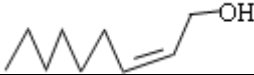
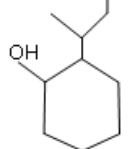
1%, 5% and 10% solutions of the *Cleome rutidosperma* essential oil were used for the anthelmintic test. Four petri-dishes were used for the three concentrations and the control. 25cm³ of each of the extract solution was placed in a petri-dish. 25cm³ of phosphate buffer saline was placed in the 4th petri-dish as control. Five adult worms, *Lumbricus terrestris*, were finally placed in each of the petri-dishes and kept at room temperature for 3h. The dead (non-motile) worms were counted and the percentage mortality was calculated.

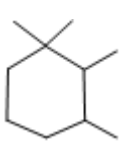
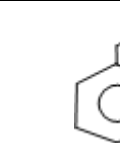
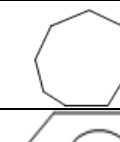
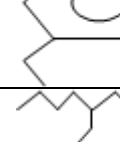
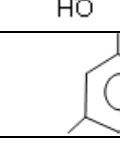
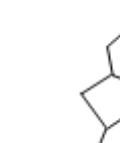
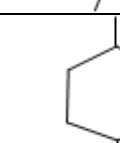
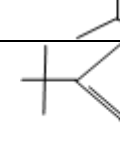
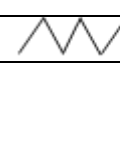
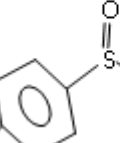
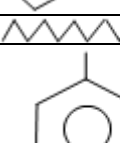
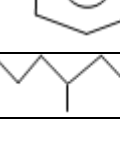


3. Results and discussion

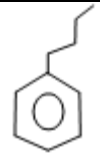
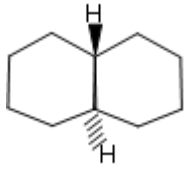
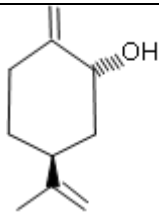
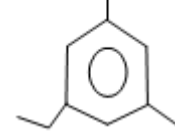
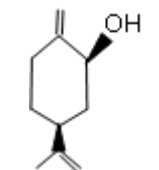
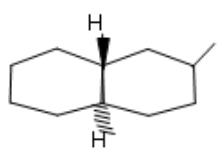

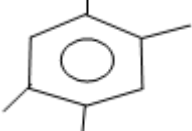
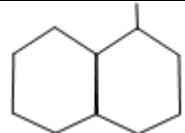
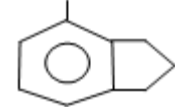

Table 1 shows the GC-MS analysis of the essential oil of the aerial part of *Cleome rutidosperma*. The oil has forty eight organic compounds, thirty seven of which are hydrocarbons. Antimicrobial sensitivity test results are contained in Table 2 while Table 3 shows the minimum inhibitory, minimum bactericidal and minimum fungicidal concentrations of the essential oil against the selected pathogenic microorganisms. The essential oil exhibited antimicrobial activity against the microbes. Table 4 shows anthelmintic activity of the *Cleome rutidosperma* essential oil. 1% solution of the essential oil killed all the worms within three hours.

Table 1: Gas Chromatography-Mass Spectroscopy Analysis of Essential Oil from *Cleome rutidosperma*

S/N	Compound Name	Retention Time (Minutes)	Molecular Formula	Relative Molecular Mass	Base Peak	Percentage composition	Chemical Structure
1	Ethylbenzene	5.137	C ₈ H ₁₀	106	91	3.01	
2	Dodecane	5.268	C ₁₂ H ₂₆	170	57	3.92	
3	O-xylene	5.431	C ₈ H ₁₀	106	91	14.93	
4	2,5-dimethyl Heptane	5.506	C ₉ H ₂₀	128	57	1.57	
5	1,2,4-trimethylcyclohexane	5.600	C ₉ H ₁₈	126	69	0.52	
6	1,1,2-trimethyl Cyclohexane	5.775	C ₉ H ₁₈	126	69	0.68	
7	1-methyl-2-propyl-cyclopentane	5.963	C ₉ H ₁₈	126	55	1.06	
8	Cis-1-ethyl-3-methyl-cyclohexane	6.038	C ₉ H ₁₈	126	97	2.29	

9	Trans-1-ethyl-3-methyl-cyclohexane	6.113	C ₉ H ₁₈	126	97	1.64	
10	p-xylene	6.313	C ₈ H ₁₀	106	91	4.80	
11	Nonane	6.682	C ₉ H ₂₀	128	57	5.44	
12	1-ethyl-2-methyl-cyclohexane	6.832	C ₉ H ₁₈	126	97	1.17	
13	Cis-1-ethyl-4-methyl cyclohexane	6.957	C ₉ H ₁₈	126	97	0.51	
14	2-methyloctahydro pentalene	7.383	C ₉ H ₁₆	124	81	2.07	
15	5,7-dimethyloctahydro coumarin	7.583	C ₁₁ H ₁₈ O ₂	182	95	0.56	
16	1-methylethylbenzene	7.820	C ₉ H ₁₂	120	105	0.72	
17	3,7,11-trimethyldodecan-1-ol	7.921	C ₁₅ H ₃₂ O	228	55	1.21	
18	Propylcyclohexane	8.014	C ₉ H ₁₈	126	83	1.60	
19	2-hexyloctan-1-ol	8.171	C ₁₄ H ₃₀ O	214	57	0.53	
20	2,6-dimethyloctane	8.415	C ₁₀ H ₂₂	142	57	2.41	
21	Trans-β,4-dimethyl cyclohexane ethanol	8.634	C ₁₀ H ₂₀ O	156	55	0.76	
22	(z),2-Nonen-1-ol	8.803	C ₉ H ₁₈ O	142	57	1.65	
23	2-(1-Methylpropyl) cyclohexanol	9.115	C ₁₀ H ₂₀ O	156	109	0.83	

24	1,1,2,3-tetramethyl cyclohexane	9.409	$C_{10}H_{20}O$	140	69	1.59	
25	Propylbenzene	9.522	C_9H_{12}	120	91	0.55	
26	Cyclooctane methanol	9.766	$C_9H_{18}O$	142	69	0.72	
27	1-ethyl-3-methyl benzene	10.097	C_9H_{12}	120	105	7.51	
28	2-butyl-octan-1-ol	10.460	$C_{12}H_{26}O$	186	57	1.02	
29	Mesitylene	10.598	C_9H_{12}	120	105	11.65	
30	1-methyl-3-(2-methylpropyl) cyclopentane	10.904	$C_{10}H_{20}$	140	55	1.22	
31	Cis-1-methyl-4-(1-methylethyl) cyclohexane	11.536	$C_{10}H_{20}$	140	55	0.71	
32	2,2,3,3,5,5,6,6-hepta methyl-3-Heptene,	11.911	$C_{14}H_{28}$	196	57	0.66	
33	Decane	13.406	$C_{10}H_{22}$	142	57	4.02	
34	1-benzenesulfinate-4-methyl,2-(1-methylethyl)-5-methylcyclohexyl ester	13.632	$C_{17}H_{26}SO_2$	294	83	0.83	
35	1-Hexadecyne	14.801	$C_{16}H_{30}$	210	81	0.85	
36	1,2,3-trimethyl benzene	14.945	C_9H_{12}	120	105	2.14	
37	4-methyl decane	15.583	$C_{11}H_{24}$	156	71	1.45	

38	Butylcyclohexane	15.884	$C_{10}H_{20}$	140	83	0.94	
39	Transdecahydro Naphthalene	17.704	$C_{10}H_{18}$	138	67	1.13	
40	Trans-p-mentha-(7),8-dien-2-ol	18.079	$C_{10}H_{16}O$	152	109	0.69	
41	1-ethyl-3,5-dimethyl benzene	19.218	$C_{10}H_{14}$	134	119	1.29	
42	Cis-p-mentha-1(7),8-dien-2-ol	19.993	$C_{10}H_{16}O$	152	109	0.89	
43	2-methyl trans decalin	24.741	$C_{11}H_{20}$	152	81	0.98	
44	Undecane	25.485	$C_{11}H_{24}$	156	57	2.28	
45	1,2,4,5-tetramethyl benzene	26.568	$C_{10}H_{14}$	134	119	0.69	
46	1-methyl decahydro Naphthalene	26.949	$C_{11}H_{20}$	152	95	0.53	
47	4-methyl-2,3-dihydro 1-H-Indene	29.526	$C_{10}H_{12}$	132	117	0.78	
48	Naphthalene	35.119	$C_{10}H_8$	128	128	0.98	

Forty eight natural products were identified in the essential oil. Thirty seven of them are hydrocarbons while the remaining eleven compounds are oxygenated. All these compounds are being reported for the first time in *Cleome rutidosperma*. P-Xylene (14.93%) has antifungal, antibacterial and antioxidant properties [18]. Some of the other identified compounds like nonane [19], trimethyldodecan-1-ol [20], 2-

nonene-1-ol [20], 1-ethyl-3-methylbenzene [21], 1, 2, 3-trimethylbenzene [21] and p-menthan-1 (7), 8-diene-2-ol [20] etc. are antifungal and antibacterial agents.

Some of these components of the essential oil have industrial applications. Nonane is a component of kerosene, fuel Additive and a component of some biodegradable detergents. P-Xylene is a major raw material for the manufacture of

terephthalic acid employed in the manufacture of terylene fibers. Ethylbenzene is used in the industry for the production of styrene which is polymerized to a common plastic known as polystyrene [22]. 1, 2, 3-Trimethylbenzene is used in jet fuel to prevent formation of solid particles which might damage

the engine while undecane is a sex attractant for a number of insects and an alert signal for a variety of ants. Propylbenzene is used as a non-polar solvent in industries including printing and dyeing of textile and manufacture of methylstyrene [23].

Table 2: Antimicrobial Sensitivity Test of Essential Oil of *Cleome rutidosperma*

Isolates	100µg/ml	50µg/ml	25µg/ml	12.5µg/ml	6.25µg/ml	Control
<i>Escherichia coli</i>	16mm	10mm	8mm	7mm	6mm	14mm
<i>Staphylococcus aureus</i>	28mm	6mm	6mm	6mm	6mm	6mm
<i>Pseudomonas aeruginosa</i>	18mm	7mm	6mm	6mm	6mm	10mm
<i>Streptococcus faecalis</i>	29mm	8mm	6mm	6mm	6mm	24mm
<i>Candida albicans</i>	28mm	24mm	10mm	8mm	6mm	20mm
<i>Aspergillus niger</i>	30mm	22mm	19mm	16mm	7mm	11mm

Table 3: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal or Fungicidal Concentration of Essential Oil of *Cleome rutidosperma*

Isolates	MIC	MBC	MFC
<i>Escherichia coli</i>	50µg/ml	100µg/ml	-
<i>Candida albicans</i>	6.25µg/ml	-	12.5µg/ml
<i>Aspergillus niger</i>	12.5µg/ml	-	25.0µg/ml
<i>Streptococcus faecalis</i>	12.5µg/ml	25µg/ml	-
<i>Pseudomonas aeruginosa</i>	25µg/ml	50µg/ml	-

Table 2 shows that *Cleome rutidosperma* essential oil has some inhibitory effect on *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, *Candida albicans* and *Aspergillus niger*. Table 3 also shows the MIC of 50 and 25µgcm⁻³ for the gram negative *E. coli* and *P. aeruginosa* respectively and 12.5µg for the gram positive *Streptococcus faecalis*. The inhibitory effect is not very pronounced for the gram negative bacteria. It has higher inhibitory effect on the gram positive bacteria. It is also further observed that the essential oil has higher antifungal than antibacterial activity. Although the biological activity is often attributed to the major constituents, the minor constituents modify their activities through antagonistic, synergistic and additive effects [24].

Table 4: Anthelmintic activity of the *Cleome rutidosperma* essential oil against adult *Lumbricus terrestris*

Concentration	No of worms	No of dead in control	No of dead in extract	% mortality
1%	5	0	5	100%
5%	5	0	5	100%
10%	5	0	5	100%

Earthworm was used for the study because it shows anatomical and physiological resemblance with intestinal worm parasites. They are therefore used as suitable models for screening of anthelmintic drugs [25]. Table 4 shows that all the adult worms died within 3hours at 1% concentration while none died in the control. This shows the essential oil is quite efficacious as an anthelmintic agent. A similar result was obtained in the chloroform fraction of the ethanol extract [1].

4. Conclusion

Essential oil from the aerial part of *Cleome rutidosperma*

contains forty eight natural products which are being identified in *Cleome rutidosperma* for the first time. The essential oil has some antimicrobial and anthelmintic activities. Some of the constituent compounds will serve as important industrial raw materials and useful drugs.

5. Conflict of interest

The authors declare no conflict of interest

6. References

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