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Frank NI Morah
Department Chemistry,
University of Calabar, Calabar,
Cross River State, Nigeria

Gloria C Apebende
Department Chemistry,
University of Calabar, Calabar,
Cross River State, Nigeria

Correspondence
Frank NI Morah
Department Chemistry,
University of Calabar, Calabar,
Cross River State, Nigeria

Chemical constituents, antimicrobial and anthelmintic activity of petroleum ether extract of aerial part of *Cleome rutidosperma*

Frank NI Morah and Gloria C Apebende

Abstract
The work is aimed at identification of chemical constituents and determination of antimicrobial and anthelmintic activity of petroleum ether extract of the aerial part of *Cleome rutidosperma*. Gc-ms analysis of the extract shows that it contains mainly eucalyptol (83.23%) as well as phytol and 4-ethyloctane. The extract has antimicrobial activity against *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Candidal albicans* and *Rhizopus nigricans*. The present work suggests that eucalyptol is responsible for the antimicrobial activity of the petroleum ether extract. The present work also shows that the extract has anthelmintic activity against *Lumbricus terrestris*. The plant will therefore serve as a source of antimicrobial and anthelmintic agents.

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

1. Introduction
Cleome rutidosperma is an annual weed which strives well in humid hot environments. It is employed in herbal medicine especially for control of parasitic skin diseases. The whole plant has antiplasmodial [1], antibacterial and diuretic activity [2]. It also has antimicrobial and anticonvulsant activity [3]. The aerial part has diuretic and laxative activity [4]. The root has wound healing activity [5], antifungal and anti-arthritic activities [6, 7]. Aqueous extract of the plant has been reported to have anthelmintic activity against the cattle exoparasite, *Haemorchus contortus* [8] while its ethyl acetate extract has antibacterial and bio enhancing activity against multidrug resistant clinical isolates [9]. Ten natural products have been identified in the chloroform fraction of the ethanol extract of the aerial part which consists of about 70% phytol [10].

2. Material and methods
2.1 Sample collection: The aerial part of *Cleome rutidosperma* was harvested within the vicinity of the Chemistry Department, university of Calabar. It was authenticated by Frank Adeoye of the herbarium unit, Botany Department, University of Calabar.

2.2 Preparation of extract: The plant material was air dried for two weeks and powdered. 30g of the powdered material was loaded into a Soxhlet extractor and extracted with petroleum ether (60-80 ^{0}C) for 2 h and was concentrated over a steam bath to give the petroleum ether extract. Separation, quantification and identification of the individual components were done with GC-MS.

2.3 GC-MS analysis: The analysis was carried out with an Agilent Hwelett-packared (789OA) with triple detector equipped with auto-injector (10µm syringe). Helium gas was used as the carrier gas. The column length is 30cm, internal diameter 0.25µm, thickness 250µm treated with phenylmethylsilox. Ion source temperature is 250 ^{0}C, pressure 16.2 psi, 1µm injection in split mode with split ratio of 1.50, with injection temperature of 300 ^{0}C. The column temperature was raised at 35 ^{0}C for 5min and changed to 150 ^{0}C at the rate of 20 ^{0}C min^{-1} and held for 5min before ionization. Microsoft solution software provided by the supplier was used to control the system and to acquire data. Identification of 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.

2.4 Agar disc diffusion method: Antimicrobial susceptibly test was carried out using the agar disc diffusion method. The following microorganisms were used: gram Negative *Salmonella typhi* and *Pseudomonas aeruginosa*; gram positive *Staphylococcus aureus* and *Streptococcus feacalis* and the fungi, *Candida albicans* and *Rhizopus nigricans*.
These microbes are clinical isolates and were maintained by the method, from the National Committee for Clinical Laboratory Standards, (NCCLS) [22]. The extract was diluted with distilled water to give solutions of 6.25, 12.5, 25, 50 and 100µgm^{-3} Mueller Hinton agar was used for both bacteria and fungi tests.
Sterilized Whatman filter paper disc were separately soaked in the solutions containing different levels of the extract. They were placed on different agar plates containing different test microorganisms. There were incubated at 37 ^{0}C for 24h. After incubation, the zone of inhibition was measured for the different plates. 100mg of doxycycline was dissolved in 100cm^3 of distilled water to give 100µgcm^{-3} solution. This was diluted to get a 10µgcm^{-3} solution of doxycycline which was used as the control.

2.5 Determination of minimum inhibitory concentration, MIC: 50, 25, 12.5, 6.25, 3.13µgcm^{-3} solutions of the extract were placed in different test tubes. 1cm^3 of water was added to each of the test tubes, peptone water (Mueller Hinton broth) 4cm^3 was added, followed by addition of 4cm^3 of 24h broth culture of the organism. The test tubes were all sealed with sterile corks and incubated at 37 ^{0}C for 24h for bacteria and 48h for fungi. Thereafter, the test tubes were observed for clearance or turbidity. The first test tube with high degree of clearance is taken as minimum inhibitory concentration (MIC) while the one preceding the MIC is regarded as minimum bactericidal concentration, MBC, for bacteria or minimum fungicidal concentration, MFC, for fungi [23, 24]. The procedure was separately carried out for the six test microorganisms.

2.6 Anthelmintic test: 1%, 5% and 10% solutions of the *Cleome rutidosperma* petroleum ether extract were used for the anthelmintic test. Four petri-dishes was used for the three concentrations and the control. 25cm^3 of each of the extract solutions were placed in three separate petri-dishes. 25cm^3 of phosphate buffer saline was placed in the 4th petri-dish as standard. Five adult worms (*Lumbricus terrestris*) were finally placed in each of the petri-dishes and kept at room temperature for 3h. The non-motile worms were counted and the percentage mortality was calculated.

3. Discussion

Table 1: Gas Chromatrography-Mass Spectroscopy Analysis of Petroleum Ether Extract of *Cleome rutidosperma*

S/N	Compound Name	Retention Time (Minutes)	Molecular Formula	Relative Molecular Mass	Base Peak	Percentage composition
1	4-ethyloctane	9.803	$C_{10}H_{22}$	142	57	4.05
2	Eucalyptol	15.934	$C_{10}H_{18}O$	154	81	83.23
3	E-3,7,11,15-tetramethyl-2-hexadecen-1-ol (phytol)	92.355	$C_{20}H_{40}O$	296	81	9.42
4	Z-3,7,11,15-tetramethyl-2-hexadecen-(phytol)	95.101	$C_{20}H_{40}O$	296	81	3.30

The petroleum ether extract contains mainly the monoterpenoid eucalyptol (83.23%), E-phytol, Z- Phytol and the saturated hydrocarbon 4-ethyloctane. Eucalyptol also known as 1, 8 – cineole has antifungal and antibacterial properties [11, 12]. It has mucolytic and spasmolytic action on respiratory track with proven clinical efficiency. It is also used against inflammatory airway diseases such as asthma and chronic obstructive pulmonary disease [13, 14, 15]. It has gastro protective effect and attenuates ulcer [16] and enhances antimicrobial activity of other antibiotics [17]. Eucalyptol has also been shown to be a powerful insect repellant against grain weevils [18].

Table 2: Antimicrobial Sensitivity Test of Petroleum Ether Extract of *Cleome rutidosperma*

Isolates	100µg/ml	50µg/ml	25µg/ml	12.5µg/ml	6.25µg/ml	Control
Salmonella typhi	10mm	7mm	6mm	6mm	6mm	15mm
Staphylococcus aureus	9mm	7mm	6mm	6mm	6mm	6mm
Pseudomonas aeruginosa	15mm	9mm	8mm	8mm	7mm	10mm
Streptococcus faecalis	13mm	8mm	7mm	6mm	6mm	23mm
Candida albicans	25mm	10mm	8mm	6mm	6mm	20mm
Rhizopus nigricans	28mm	8mm	6mm	6mm	6mm	9mm

The fungi *Rhizopus nigricans* and *Candida albicans* have the zone of inhibition of 28 and 25mm which is higher than those of the bacteria.

Table 3: Minimum inhibitory concentration and minimum bactericidal/fungicidal concentration of petroleum ether extract of *Cleome rutidosperma.*

Isolate	MIC	MBC	MFC
Zhizopus nigricans	6. 25µgcm^{-3}	-	12.5µgcm^{-3}
Candida albicans	1.5 µgcm^{-3}	-	3.13µgcm^{-3}
Staphylococcus aureus	3.13 µgcm^{-3}	6.25µgcm^{-3}	-
Streptococcus faecalis	1.5 µgcm^{-3}	3.13µgcm^{-3}	-
Pseudomonas aeuruginosa	1.5 µgcm^{-3}	3.13µgcm^{-3}	-
Salmonella typhi	12.5 µgcm^{-3}	25 µgcm^{-3}	-

Table 3 shows that minimum inhibitory concentration of 3.13 and 12.5µgcm^{-3} are observed for the gram negative *Streptococcus faecalis* and *Salmonella typhi* respectively while it is 1.5µgcm^{-3} for the gram positive *Streptococcus faecalis* and *Pseudomonas aeruginosa*. This shows that the gram positive bacteria are more sensitive to the extract. It will be a more effective drug for the gram positive than the gram negative bacteria. MIC of 1.5 and 6.25 were also observed for the fungi *Candida albicans* and *Rhizopus nigricans* respectively showing that it is also a good antifungal agent.

The corresponding author monitored treatment of *tinea* infection which had spread all over the foot with *Cleome rutidosperma* by a traditional medical practitioner in Calabar. The aerial part of *Cleome rutidosperma* was ground and mixed with vaseline to form a poultice. The poultice was applied to the affected area after bath in the morning and evening. This effected healing within one week. In the absence of vaseline, kerosene or any other liquid hydrocarbon could be used in poultice formation. This points to the fact that the antifungal agent is non-polar and soluble in hydrocarbon solvents. About 83% of the petroleum extract of *Cleome rutidosperma* is eucalyptol (1, 8 – cineole), a known antifungal agent [7]. The present work shows that the petroleum extract of the aerial part of *Cleome rutidosperma* has antifungal activity against *Candids albicans* and *Rhizopus nigricans.* It is therefore conceivable that the principle antifungal agent of *Cleome rutidosperma* is *eucalyptol.* Since *eucalyptol* also has antibacterial and wound healing properties [5, 17], it has added advantage of taking care of secondary bacterial infections and skin ruptures that might be associated with fungal infections.

Earthworms are used for screening of anthelmintic drugs because they show anatomical and physiological resemblance with intestinal worm parasites [19].

Table 4: Anthelmintic activity of *Cleome rutidosperma* petroleum ether extract against adult *Lumbricus terrestric*

Concentration	No. of Worms	No. of Dead Worms in Control	No. of Dead Worms in Extract	% Mortality
1%	5	0	4	80%
5%	5	0	5	100%
10%	5	0	5	100%

Table 4 shows that only 80% of adult *Lumbricus terrestris* died after three hours at 1% concentration. 100% died at 5% and 10% concentrations of the extract while none died in the control. Phytol is known to have a strong anthelmintic activity against schistosomiasis [20].

Eucalyptol is also known to have anthelmintic activity against monogenea infections of fish [21]. Eucalyptol and phytol are therefore responsible for the observed anthelmintic activity of the petroleum ether extract of *Cleome rutidosperma.*

4. Conclusion

The petroleum ether extract consists of over 83% eucalyptol which is being identified for the first time in *Cleome rutidosperma.* Eucalyptol is being reported as the main antimicrobial and anthelmintic principle of the petroleum ether extract. Eucalyptol can therefore be developed into anthelmintic, antibacterial and antifungal drugs.

5. References

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