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CHEMICAL COMPOSITION AND ANTIBACTERIAL PROPERTIES OF THE ESSENTIAL OIL OF *CERATOTHECA TRILOBA* (BERNH.) HOOK.F.

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Abstract

The chemical composition and antibacterial properties of the essential oil from *Ceratotheca triloba* (Bernh.) Hook. f. were studied. In all 51 chemical compounds were identified in the oil by gas chromatography (GC/MS). This represented 99.7% of the oil. The major compounds present in the essential oils were phytol (23.47%), dibutyl phthalate (6.12%), cyclopenta [c]pyran-4-carboxylic acid, 7-methyl-, methyl ester (5.73%), 1,3-dimethylbenzene (3.52%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (4.84%), 9-octyleicosane (4.10), (4-methylphenyl)(phenyl) methanone (3.42%), (E)-1-(2,6,6-trimethylcyclohexa-1,3-dien-1-yl) but-2-en-1-one (3.16%), 3-methyl heptadecane (3.03%), n-hexadecanoic acid (2.67%), hexadecane (2.27%). The essential oil was tested against the following bacteria: *Staphylococcus aureus, Pseudomonas* sp., *Escherichia coli, Klebsiella* sp. and *Proteus* sp. Strong antimicrobial activity exhibited against *Staphylococcus aureus, Pseudomonas* sp., *Escherichia coli* and *Proteus* sp. but not against *Klebsiella* sp. The minimum inhibitory concentration (MIC) of extracted oil ranged from 100 and 280 µg/ml. Standard antibiotic discs with *Chloramphenicol* were used to compare with positive controls.

Numerous plants have been routinely screened for their potential medicinal use as alternative remedies for the treatment of many infectious diseases (Agunu *et al.* 2005). In particular, the antibacterial and antiviral activities of plant oils and extracts have formed the basis of many investigations, including food preservation, pharmaceuticals, alternative medicine and natural therapies (Friedman *et al.* 2002). Recently, there has been a trend towards using natural products and phytochemicals in the medicine and food industries. This was primarily to overcome antibiotic resistance and to increase the shelf life of products. Plant essential oils primarily serve as a defense against pathogens and pests (Oxenham 2003).

The family, Pedaliaceae, which comprises 13 genera and 70 species, is exclusively tropical and usually occurs in dry habitat of the old world. *Ceratotheca triloba* (Bernh.) Hook. f. is one of the four species found in South Africa. In a previous study, three structurally similar anthraquinones; 9, 10-anthracenedione, 1- hydroxy-4-methylanthraquinone and 5, 8- dimethoxy-2, 3, 10, 10a-tetrahydro-1H, 4aH-phenanthrene-4, 9-dione, and one steroid; androst-5-ene-3, 17, 19-triol were isolated from the root extracts of *C. triloba* (Mohanlall *et al.* 2011). Anthraquinones are an important group of natural products that are found in plants (Korunaglo *et al.*1992, Bajaj 1999). Derivatives of the anthraquinone molecule exhibit various pharmacological and biological activities, including anticancer, antibacterial, antitrypanosomal and antineoplastic (Baguley 1991, Monneret 2001, Dzierzbicka and Kolodziejezyk 2005, Kovacevic and Grubisic 2005 and Preobrazhenskaya *et al.* 2006). Recent studies have shown that the anthraquinones from fresh roots of *C. triloba* inhibit the activity of the human topoisomerase II enzyme which transforms super coiled DNA to linear DNA. *C. triloba* is used in traditional medicine to treat a variety of illnesses including menstruation, stomach cramps, nausea, fever and diarrhea (Tredgold 1986). The chemical composition of the volatile oil extracted from leaves of *C. triloba* using GC/MS analyses as well as their antibacterial properties were investigated.

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Fresh leaves of *C. triloba* were collected from Umhlanga, Durban, South Africa. After identification, a voucher specimen was deposited in the Herbarium, School of Life Sciences, University of KwaZulu-Natal, (Accession number 11701).

Oil was extracted from 500 g fresh leaves for 5 hrs by steam distillation using the Clevenger system. The essential oil was transferred into a stoppered tube and stored at 0°C in air-tight containers, after drying over anhydrous sodium sulfate. Volatile compounds were identified by gas chromatography/mass spectrometry (GC/MS) in an Agilent gas chromatography N6890 fitted with a HP-5MS fused silica column (5% phenyl methyl polysiloxane 30 m × 0.25 mm, film thickness 0.25 µm), interfaced with an Agilent 5975C VLMSD with triple axis mass detector. Oven temperature was 60 - 230°C at 5°C/min. The injection temperature was 280°C. Helium, the carrier gas, was supplied at 1.0 ml/min. The split ratio was 1 : 10, and the split flow 10 ml/min. Mass scans ranged from 50 to 500 amu. Aliquots of 1 µl sample (dissolved in hexane) were injected into the system. Identification of the compounds was based on the comparison of retention indices, mass spectra and the NIST spectrometer data bank, as well as comparison with data in the literature.

Antibacterial activity of the plant oil was tested against several test bacteria *viz. Staphylococcus aureus* (MTCC 3160), *Pseudomonas aeruginosa* (MTCC741), *Escherichia coli* (MTCC40), *Klebsiella pneumoniae* (MTCC3384) and *Proteus mirabilis*, (MTCC425). Bacteria were obtained from Institute of Microbial Technology, Chandigarh (IMTC), India.

Antibacterial assays were determined using the disc diffusion method (Murray *et al.* 1995). Two concentrations of essential oil samples were prepared: 1 mg/ml and 2 mg/ml in 10% DMSO. The bacterial suspension (100 µl) was adjusted at 1×10^6 CFU/ml of bacteria which was spread by a sterile glass rod on Nutrient Agar (NA). Filter paper discs (Whatman No.1, 5 mm in diameter) were impregnated with 4 µl of the essential oil, placed on inoculated plates, incubated at 27 ± 2°C for 24 hrs, and inhibition zones were measured. Standard antibiotic discs containing 30 µg of *Chloramphenicol* (Himedia, India) were used as positive and DMSO as negative controls. The experiments were performed in triplicate.

Minimum inhibitory concentration was determined by the broth dilution technique (Atta-ur-Rahman *et al.* 2005). The essential oil was prepared at different concentrations (25, 50, 100, 150 and 200 µl/ml) in the nutrient broth. Using micropipette, 0.05 ml of the 18 hrs old bacterial broth (10^6 CFU/ml) culture was introduced into each of the test tubes with different concentrations of the volatile oil. A set of tubes containing only growth medium, as well as each of the test bacteria, were set as controls. All tubes were incubated at 27 ± 2°C for 30 hrs. The MIC was the lowest concentration of volatile oil that prevented bacterial growth. Standard antibiotic *Chloramphenicol* served as a positive control.

The chemical analyses of essential oil are presented in Table 1. The yield of the essential oil of *C. triloba* was 0.38%. In total, 51 compounds were identified, representing 99.7% in the oil. The main compounds were phytol (23.47%), dibutyl phthalate (6.12%), cyclopenta [c]pyran-4-carboxylic acid, 7-methyl-, methyl ester (5.73%), 1,3-dimethylbenzene (3.52%), 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol (4.84%), 9-Octyleicosane (4.10%), (4-ethylphenyl) (phenyl) methanone (3.42%), (E)-1-(2,6,6-trimethylcyclohexa-1,3-dien-1-yl)but-2-en-1-one(3.16%), 3-methylheptadecane (3.03%), n-hexadecanoic acid (2.60%), hexadecane (2.27%) and 4,7-dimethyl-1-propan-2-yl-1,2,3,5,6,8a-hexahydronaphthalene (2.04%).

Antibacterial activities against Gram-positive and Gram-negative bacteria of the essential oil of *C. triloba* are presented in Table 2.

Table 1. Chemical constituents of the essential oil of *Ceratotheca triloba*.

Sl.No.	Compounds	RT	% of components	RI
1	1,2-dimethylcyclohexan	3.29	0.50	
2	Ethylcyclohexan	3.33	0.63	829
3	Ethylbenzene	3.70	0.75	846
4	1,3-dimethylbenzene	3.82	3.52	864
5	Nonane	4.21	1.12	900
6	1-hepten-3-ol	5.70	0.78	869
7	1,2,3-triméthylbenzène	6.09	0.58	1001
8	3,8-dimethyldecane	6.15	0.61	1063
9	1-methyl-2-pyrrolidinone	7.40	0.95	1045
10	4-(methylthio)-1-butene	7.54	0.80	
11	1-phenylethanone	7.77	0.81	1065
12	3,7-dimethyl-1,6-octadien-3-ol	8.56	1.22	1100
13	Naphthalene	10.80	0.78	1179
14	4-methylbenzoic acid, methyl ester	11.54	1.19	
15	1-methylcyclopentene	15.31	0.85	660
16	(E)-1-(2,6,6-trimethylcyclohexa-1,3-dien-1-yl)but-2-en-1-one	16.03	3.16	1440
17	Tetradecane	16.32	1.07	1413
18	3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	18.54	0.97	1484
19	Pentadecane	18.77	0.66	1500
20	1-fluoro-2,4-dinitrobenzene	19.21	1.51	1446
21	4,7-dimethyl-1-propan-2-yl-1,2,3,5,6,8a-hexahydronaphthalene	19.45	2.04	1548
22	Heptacosane	20.45	0.57	1774
23	Cyclopenta[c]pyran-4-carboxylic acid, 7-methyl-, methyl ester	20.99	5.73	
24	Hexadecane	21.11	2.27	1612
25	Heptadecane	24.32	0.52	1700
26	(4-methylphenyl)(phenyl)methanone	24.63	3.42	
27	3-methylheptadecane	24.86	3.03	1768
28	8-methylheptadecane	25.47	2.20	1746
29	2,6,10,14-tetramethylhexadecane	25.67	0.65	1789
30	Hexadecane			1600
31	Cis-3-hexenyl cinnamate	27.19	2.24	1897.4
32	Nonadecane	27.50	0.82	1910

(Contd.)

33	Docosanoic acid, methyl ester	28.03	0.71	
34	n-hexadecanoic acid	28.65	2.60	1938
35	Dibutyl phthalate	28.78	6.12	1940
36	3-methyldecane	28.89	1.36	
37	7-propyltridecane	29.44	0.48	
38	Henicosane	31.28	1.01	2100
39	Phytol	31.54	23.47	2102
40	9 12 15-octadecatrienoic acid (z z z)-	31.96	0.73	2199
41	2-methyl-1-hexadecanol	32.08	0.63	
42	3,7,11,15-tetramethyl-2-hexadecen-1-ol	32.27	4.84	2066
43	Docosane	32.57	1.11	2200
44	Butyl heptadecyl sulfite	33.06	0.56	
45	17-n-hexadecyltetratriacontane	34.76	0.57	
46	Pentacosane	37.97	1.16	2500
47	1,2-benzenedicarboxylic acid, diisooctyl ester	38.81	1.72	
48	Hexacosane	39.66	0.48	2600
49	9-octyleicosane	41.73	4.10	2000
50	Di-n-decylsulfone	42.55	0.80	
51	Pentacosane	47.53	1.10	

Table 2. The diameter of the antibacterial activity zone (mm) of the essential oil of *Ceratotheca triloba*.

Test bacteria	Essential oil		Positive control	MIC µg/ml
	1 mg/ml	2 mg/ml		
Escherichia coli	8	15	30	156
Staphylococcus aureus	12	22	35	100
Klebsiella sp.	-	-	25	
Pseudomonas sp.	6	11	32	150
Proteus sp.	7	10	30	280

Values are diameter of zone of inhibition (mm). Positive control = Standard antibiotic disc paper 5 mm contained 30 µg of chloramphenicol.

The essential oil was active against *Staphylococcus aureus*, *Pseudomonas* sp., *E. coli* and *Proteus* sp. but not against *Klebsiella* sp. The maximum zone of inhibition (22 mm) in this study was against *Staphylococcus aureus*. The MIC values validated the antibacterial activity against the tested bacteria. The MIC values of oil ranged from 100 to 280 µg/ml (Table 2). Yoshihiro *et al*. (2005) demonstrated that phytol possessed antibacterial and antioxidant activity against the growth of *S. aureus.* Phytol, an important plant metabolite, is used in shampoos, toilet soaps and cosmetics. Phytol has been shown to exhibit anticancer, antidiuretic and antimicrobial activity

(Raman *et al.* 2012). Phytol is also important in the processing of glucose and in the activation of enzymes within the body and therefore has strong positive effects on insulin level (Liljenberg 1971). This suggests that the inclusion of phytol in the diet could possibly stimulate the metabolic functions of those patients with Type 2 diabetes (March 2002). Phytol, a branched-chain fatty alcohol which is a component of the chlorophyll pigment, is released only in the ruminants' digestive system, presumably by bacteria present in their gut (Hansen 1966). It is present in the adipose tissues of animals and in dairy products at relatively high levels. Brown *et al.* 1993 and Gloerich *et al.* 2005 reported increased levels of phytol and its metabolites (*viz*., phytenic, phytanic and prystanic acids) in plasma and liver of mice that were fed a phytol enriched diet. They suggested that absorbed phytol is transported to the liver and metabolized into phytanic acid through three enzymatic steps, which are believed to be under the control of PPARα (Brink *et al.* 2005). In humans and mammals, phytanic acid appears as an oxidized product of phytol following ingestion of fat-containing foods of animal or plant origin. Subsequently phytanic acid is absorbed into the small intestine. Phytol is therefore a precursor of the natural rexinoidphytanic acid that triggers RXR and activates the full spectrum of PPARs (Heim *et al.* 2002). The volatile oil from *C. triloba* appears to be a suitable, potential candidate for use as an antimicrobial agent. This study has clearly demonstrated that the African medicinal plant *C. triloba* produces essential oil that possesses medicinal and antibacterial properties.

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