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Full Length Research Paper

Phytochemical composition and biological activities of essential oil of *Rhynchosia minima* (L) (DC) (Fabaceae)

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The major constituents, antimicrobial activities against 12 bacterial and 4 fungal species and the antioxidant activity of Rhynchosia minima essential oil were determined. The yield of the oil was 0.21%. The main components of the oil included isopropyl toluene, camphene and O-cymene. The essential oil exhibited marked activity against Escherichia coli, Staphylococcus aureus and Candida albicans, but was not active against Clostridium perfringens up to the concentration of 100 µg/ml. The essential oil also exhibited anti-oxidant activity. The significant antimicrobial and antioxidant activities of R. minima oil suggests that it could serve as a source for compounds with therapeutic potential.

Key words: *Rhyncosia minima,* essential oil, antibacterial, anti-fungal, anti-oxidant, phytoconstituents.

INTRODUCTION

Essential oils are produced by different types of plants to serve as plant defense mechanisms against predation by microorganisms, insects and herbivores or for reproductive purposes, for example, to lure bees for pollination (Theis and Lerdau, 2003; Dudareva and Negre, 2005). These oils can be extracted from different parts of the plant and are made up of different chemical compounds that occur naturally in the plant such as alcohol, hydrocarbons, phenols, aldehydes, esters, terpenes and ketones which are some of the major components (Theis and Lerdau, 2003). However, the composition of essential oils varies from plant species to another.

Essential oils have been demonstrated to have a wide range of biological activities and are also used in the manufacture of perfumes, deodorants and soaps (Mandalari et al., 2007). Over the last decade, the use of medicinal plants and their essential oils in particular has increased due to the globalization and increase in international trade, but also because they are environmentally friendly and readily available. However, only few African plants are used for the production of the oils for commercial or industrial purposes.

In our search for essential oils for the medical, cosmetics, perfumery, fragrance or flavors industries, *Rhynchosia minima* was selected based on its traditional use in skin conditions and to alleviate boils in Zimbabwe. *R. minima* belongs to the Fabaceae family of plants. It is a glabrous to somewhat pubescent, perennial, twining or sub-erect legume. This plant has a world wide distribution and in Zimbabwe it is found in most parts of the country. It grows well on sandy soil on a mixed altitude of ±1600

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Table 1. Major identified constituents of *Rhynchosia Minima* essential oil and their relative proportion in the oil.

GC peak number	Component	Composition (%)	Retention time
1	α-Pinene	1.287	5.38
2	Isopropyl toluene	33.660	2.57
3	Camphene	19.304	3.23
4	2-ß-Pinene	2.858	6.58
5	Myrcene	0.966	6.97
6	O-Cymene	26.383	7.83
7	Limonene	14.128	7.97
8	α-Terpinolene	1.415	9.42

feet. The aim of the present study was to determine the biological effects of the essential oil of *R. minima* using conventional methods and to analyze its composition using gas chromatography-mass spectrometry (GC-MS).

MATERIALS AND METHODS

Plant material collection and isolation of the essential oil

The plant material was collected in the district of Harare, Zimbabwe with the authorization of the Zimbabwean government and in agreement with the United Nation Convention on Biodiversity. The voucher specimens were deposited at the Herbarium of the Department of Botany, in the University of Zimbabwe. Fresh Leaves (1000 g) were subjected to steam distillation for approximately 3 h using a Clevenger-type apparatus. The essential oil was dried over anhydrous sodium sulphate to remove any traces of water and, after filtration, stored at approximately 4°C until tested and analyzed.

Gas chromatography and mass spectroscopy analysis

GC-MS analysis was performed as previously described using a Hewlett Packard 6890 Gas Chromatograph (Adams, 2001).

Determination of antibacterial activity of essential oils

The antibacterial activity of the essential oil was determined by the hole plate method as previously described (Gundidza et al., 1993). The bacterial organisms used in the present study included: *Acenotobacter calcoacetilus* (NCIB 8250), *Bacillus subtilis* (NCIB 3610), *Citrobacter freundii* (NCIB 11490), *Clostridium perfringens*, *Clostridium sporogenes* (NCIB 10696), *Escherichia coli* (NCIB 8879), *Klebsielia pneumoniae* (NCIB 4184), *Proteus vulgaris* (NCIB 4175), *Pseudomonas aeruginosa* (NCIB 950), *Salmonella typhii*, *Staphylococcus aureus* (NCIB 6751) and *Yersinia enterocolitica* (NCIB 10460).

Anti-fungal testing

The antifungal activity of the essential oil was determined as previously described (Gundidza et al., 1993). The level of inhibition was calculated from the formula: Percentage inhibition = [(C- T)/C] x 100; Where C is the mean dry weight of fungal cells from the control flasks, and T is the mean dry weight of fungal cells from the test flasks. Four fungal species were used for testing were, *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus* and *Penicillium notatum*. All the organisms were obtained from the University of Zimbabwe, Department of Pharmacy.

Determination of antioxidant activity of essential oil

The antioxidant activity of the essential oil was determined as previously described (Gundidza et al., 1993). Absolute alcohol was used as a negative control and the ascorbic acid (10 mg/ml) was used as a positive control.

RESULTS AND DISCUSSION

The yield of the essential oil was 0.18% (v/w). The major phytochemical components of essential oil from *R. minima* from Harare, Zimbabwe, were isopropyl toluene, O-cymene, camphene, limonene, 2- β-pinene, α-terpinolene, α-pinene and myrcene (Table 1). This profile is similar to that in *Melaleuca alternifolia* essential oil which was also shown to have antibacterial activities. It can then be hypothesized that the mode of bacterial inhibition is similar to that of *M. alternifolia* oil (Cox et al., 2000).

The essential oil of *R. minima* exhibited antibacterial activity against all the bacterial species tested except *Clostridium perfringens* and *Klebsiella pneomoniae* (Table 2). These activities may be attributed to the presence of major compound such as O-cymene, and other compounds found described in the present study. Enantiomers of α-pinene, 2-β-penene and limonene have been suggested to have a strong antibacterial activity (Tzakou et al., 2001; Filipowicz et al., 2003). It has been demonstrated that α-pinene and β-pinene are able to destroy cellular integrity and thereby inhibit respiration and ion transport processes. They also increase the membrane permeability in yeast cells and isolated mitochondria (Andrews et al., 1980). The possible mechanisms of other essential oil components such as trans-caryophyllene, limonene, camphene, (-)-bornylacetate, tridecane and α-humulene have not yet been elucidated. However they may act synergistically.

The essential oil exhibited significant activity against all the fungal species (Table 3). It showed comparable acti-

Table 2. Antibacterial activity of *Rhynchosia Minima* essential oil at different concentrations.

Bacterial species	Inhibition zone diameter (mm)					
	NC	10 µg/ml	20 µg/ml	50 µg/ml	100 µg/ml	Positive control
A. calcoaceticus	0	0	5.9	5.9	7.0	18.9
B. subtilis	0	0	0	5.1	7.5	16.8
C. freundii	0	5.0	6.6	8.1	10.0	17.0
C. perfringens	0	0	0	0	0	16.2
C. sporogenes	0	0	0	0	4.5	16.9
E. coli	0	8.7	9.8	12.2	18.2	20.7
K. pneumoniae	0	0	0	0	0	19.0
P. vulgaris	0	4.2	4.9	6.0	7.0	17.8
P. aeruginosa	0	4.1	6.2	8.0	11.2	14.5
S. typhii	0	0	0	4.6	5.2	17.2
S. aureus	0	6.1	8.0	9.8	12.3	16.1
Y. enterocolitica	0	5.4	6.0	9.1	13.0	17.9

NC = Negative control

Table 3. Antifungal activity of *Rhyncosia minima* essential oil.

Fungal species	Percentage Inhibition					
	NC	2.5 µg/ml	5 µg/ml	10 µg/ml	100 µg/ml	*Clotrimazole 100 µg/ml
C. albicans	0	46.3	53.7	72.1	84.8	79.7
A. niger	0	37.5	45.4	45.4	49.4	78.2
A. flavus	0	42.3	68.1	70.3	75.4	76.2
P. notatum	0	24.6	36.7	45.9	56.5	69.2

*Positive control.

vity with the positive control against *C. albicans* and *A. flavus.* The high antifungal activity may be attributed to the presence of the said chemical components in the essential oil. O-Cymene and limonene have been shown to have strong antifungal properties (Filipowicz et al., 2003). The mechanism of antifungal activity of this essential oil is still unknown. However, a recent study on the induced damage to cell membrane structure of yeast and isolated mitochondria which suggests that phytoconstituents from the essential oil of *S. portulacastrum* for example are likely to disrupt the permeability barrier of cell membranes and thereby inhibit respiration (Helander et al., 1998).

The essential oil from *R. minima* showed anti-oxidant activity, with a mean zone of color retention of 6.8 mm compared to 10 mm for the positives control. Monoterpenes found in this essential oil may act as radical scavenging agents. It seems to be a general trend that the essential oils which contain monoterpene hydrocarbons, oxygenated monoterpenes and/or sesquiterpenes have greater antioxidative properties (Tepe et al., 2004; Mau et al., 2003).

In conclusion our findings suggest that the essential oil from *R. minima* has potential as antibacterial, antifungal and antioxidant agent and may be useful in the pharmaceutical and cosmetics industries. More research is needed to ascertain this.

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REFERENCES

Adams RP (2001). Identification of Essential Oil Components by Gas Chromatography/ Mass Spectroscopy. Allured Publishing, Carol Stream, IL, USA.

Andrews RE, Parks LW, Spence KD (1980). Some effects of Douglas fir terpenes on certain microorganisms. Appl. Environ. Microbiol., 40: 301-304.

Cox SD, Mann CM, Markham JL, Bell HC, Gustafson JE, Warmington JR, Wyllie SG (2000). The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (Tea tree oil). J. Appl. Microbiol. 88: 170-175.

Dudareva N, Negre F (2005). Practical applications of research into the

regulation of plant volatile emission. Curr. Opin. Plant. Biol. 8(1): 113-118.
Filipowicz N, Kamiński M, Kurlenda J, Asztemborska M (2003). Antibacterial and antifungal activity of Juniper Berry oil and its selected components. Phytother. Res. 17: 227-231.
Gundidza M, Deans SG, Kennedy AI, Mavi S, Waterman PG, Gray AI (1993). The essential oil from *Heteropyxis natalensis* Harv. Its antimicrobial activities and phytoconstituents. J. Sci. Food Agric. 63: 361-364.
Helander IM, Alakomi HL, KyostiL K, Mattiala-andholm T, Pol I, Smid EJ, Gorris GM, von Wright A (1998). Characterization of the action of selected essential oil components on Gram-negative bacteria. J. Agric. Food Chem. 46: 3590-3595.
Mau JL, Lai EYC, Wang NP, Chen CC, Chang CH, Chyau CC (2003). Composition and antioxidant activity of the essential oil from *Curcuma zedoaria*. Food Chem. 82: 583-591.
Mandalari G, Bennett RN, Bisignano G, Trombetta D, Saija A, Faulds CB, Gasson MJ, Narbad A (2007). Antimicrobial activity of flavonoids extracted from bergamot (*Citrus bergamia* Risso) peel, a byproduct of the essential oil industry. J. Appl. Microbiol. 103(6): 2056-2064.
Tepe B, Donmez E, Unlu M, Candan F, Daferera D, Vardar-Unlu G, Polissiou M, Sokmen A (2004). Antibacterial and antioxidative activities of the essential oils and methanol extracts of *Salvia cryptantha* (Montbret et Aucher ex Benth.) and *Salvia multicaulis* (Vahl). Food Chem. 84: 519-525.
Theis N, Lerdau M (2003). The evolution of function in plant secondary metabolites. Int. J. Plant. Sci. 164(3): S93-S102.
Tzakou O, Pitarokili D, Chinou IB, Harvala C (2001). Composition and antimicrobial activity of essential oil of *Salvia ringens*. Planta Med. 67: 81-83.