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Full Length Research Paper

Biological activity and phytoconstituents of essential oil from fresh leaves of *Eriosema englerianum*

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Essential oil was extracted from fresh leaves of *Eriosema englerianum* by hydrodistillation and its major phytoconstituents determined by GC-MS. The major phytoconstituents were O-cymene, terpinolene and ascaridole with a yield of 0.28%. Antimicrobial activity of the oil was tested against nine human bacterial pathogens and three opportunistic fungi (*Candida albicans*, *Aspergillus niger* and *Aspergillus flavus*). The essential oil showed anti-oxidant activity with mean zone of colour retention of 14.4 mm and good antibacterial and antifungal activities. Results of present study revealed that the fresh leaves of *E. englerianum* yield essential oil which contains medicinal properties.

Key words: Antifungal, antibacterial, antioxidant, essential oil.

INTRODUCTION

Eriosema Englerianum Harms also known as Blue bush or Mashona fire bean is a member of the Fabaceae family of plants. This family includes herbs, vines, shrubs, trees, and lianas found in both temperate and tropical areas (Inngjerdingen et al., 2004). They comprise one of the largest families of flowering plants, numbering 630 genera and 18,000 species (Kakudidi, 2004). In Zimbabwe, as in many developing countries, plants represent the principal means of therapy in traditional medicine and such practice plays vital role in health care system especially in rural areas.

In Zimbabwe, *E. englerianum* is mostly found in Mashonaland, between Chegutu and Gazema and Norton, near Harare. This plant is also found in limited areas in the Democratic Republi c of Congo (DRC), Zambia, Malawi, South Africa and Mozambique. The roots are used in combination with other plants such as *Vigna ungiculata* and *Terminalia sericea* to treat bilharziosis. In South Africa, Zulu traditional health practitioners have claimed that the roots of *E. kraussianum* N. E. Br. (Fabaceae) and other *Eriosema* species (known in Zulu under the umbrella name of "uBangalala") are effective remedies for the treatment of erectile dysfunction (ED) and/or impotence (Ojewole et al., 2005). However, no study has been conducted on the essential oil from this plant.

Essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties (Kordali et al., 2005; Marwah et al., 2007; Esmaeili et al., 2008). In the present study, fresh leaves of *E. englerianum* were collected and essential oil was prepared by hydrodistillation. The isolated oil was then studied for biological activities including antibacterial, antifungal and antioxidant activities while gas chromatography mass spectrometry (GC-MS) was used for the determination of the major compounds contained in the oil.

MATERIALS AND METHODS

Plant material collection

The plant material was collected in the districts of Zimbabwe with the authorization of the Zimbabwean government and in agreement

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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.

Essential oil extraction

Fresh Leaves (1000 g) were subjected to steam distillation for approximately 6 h using a Clevenger-type apparatus. The yield obtained was 0.28% v/w. The essential oil was dried over anhydrous sodium sulphate and, after filtration, stored at 4°C until tested and chemically analyzed. The essential oil was subjected to GS/MS analysis to identify the phytoconstituents.

Gas chromatography and mass spectroscopy analysis

A wet needle method was used to analyze the essential oil by means of a Hewlett Packard 6890 Gas Chromatograph. The temperature of the injection port was set at 220°C while the pressure at the inlet was maintained at 3.96 psi. A HP-5MS (cross linked 5% Phenyl Methyl Siloxane) column (30 m x 0, 25 mm x 0.25 µm film thickness) was temperature programmed from 60 to 150°C at 3°C per min after a 3.5 min delay. Helium was used as a carrier gas at 0.7 ml/min. Mass spectra were recorded by a HP 5937 series Mass Selective Detector (MSD). The detector sensitivity was set at 1.28 x 10-10 g; the injection volume at 0.1 µl; the air flow rate at 15 ml/min and the hydrogen flow rate at 15 ml/min. The sample was dissolved in CH_2Cl_2 and a split injection technique was used. Identification of oil components was achieved based on their retention indices (determined with reference to a homologous series of normal alkanes), and by comparison of their mass spectral fragmentation patterns (NIST database/ChemStation data system) (Magwa et al., 2006).

Determination of the antibacterial activity of the essential oils

Screening of essential oils for antibacterial activity was done by the disk diffusion method (Samie et al., 2005). The essential oils were dissolved in 10% aqueous dimethylsulfoxide (DMSO) with Tween 80 (0.5% v/v for easy diffusion) and sterilized by filtration through a 0.45 µm membrane filter. Paper disc moistened with aqueous DMSO was placed on the seeded Petri plate as a vehicle control. A standard disc containing streptomycin (25 µg/disc) was used as reference control. All Petri dishes were incubated at 37°C for 18 h-24 h and the zone of inhibition was measured with a calliper. Studies were performed in triplicate, and mean value was calculated. Eight different bacterial species were used including *Staphylococus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumoninae, Proteus vulgaris, Chlostridium sporogens* and *Acinotobacter calcoaceticus*.

Determination of antifungal activity of essential oils

The antifungal activity of the essential oils was determined as previously described (Magwa et al., 2006). Clotrimazole cream (10 mg/g) was dissolved in 10 ml of absolute ethanol and used as positive control. The level of inhibition was calculated from the formula:

Percentage inhibition = [(C- T)/C] x 100

Where C is the mean dry weight of hyphae from the control flasks, and T is the mean dry weight of hyphae from the test flasks. The three fungal species used for antifungal testing are *Candida albicans, Aspergillus flavus* and *Aspergillus niger.* All the organisms were obtained from the University Of Zimbabwe Department Of Pharmacy.

Determination of antioxidant activity of essential oils

The antioxidant activity of the essential oils was determined as previously described (Magwa et al., 2006). Briefly, a medium composed of technical agar, β-carotene (sigma) and linoleic acid (sigma) was prepared and poured in Petri dishes and kept in the dark. After the medium has set, holes (4 mm diameter) were punched using a borer and the oil (25 µl) was transferred into the holes and the Petri dishes were then incubated at 45°C for 4 h. A zone of colour retention around the hole following incubation noted essential oils with antioxidant properties. The zone diameter was measured using vernier calipers after the oil has been withdrawn from the hole. Absolute alcohol was used as a negative control and the ascorbic acid (10 mg/ml) was used as a positive control.

Qualitative and quantitative analysis

Most photoconstituents were characterized by gas chromatography by comparison of their GC retention indices (RI) with those found in literature (Pratheung et al., 2006). Identification of volatile components was further performed by matching their mass spectra with HP 5937 series Mass selective detector. Quantitative analysis of each volatile component in percent was performed by peak area normalization in duplicate.

RESULTS AND DISCUSSION

Essential oils and extracts have been used for many thousands of years in food preservation, pharmaceuticals, alternative medicine and natural therapies (Cimanga et al., 2002; Sylvestre et al., 2006). Essential oils are potential sources of novel antimicrobial compounds (Simic et al., 2004) especially against bacterial pathogens. Our study has revealed three major compounds from the essential oil from leaves of *E. engleria*num with O-cymene containing the highest percentage (88.49%) with a retention time of 6.664 min, followed by terpinolene (7.858%) with retention time of 13.770 min and ascaridole (3.651%) with a retention time of 16.395 min. O-Cymene has also been found to be present in the essential oil from *Pteronia incana* by Magena and Muyima (1999). Terpinolene and ascaridole were found to be of lower percentage in the present study. Other compounds have been isolated from other parts of plants from the same genera particularly *E. tuberosum* including three new phenolic glycosides named eriosemasides A - C (Ma et al., 1999).

The anti-bacterial activity of essential oils against nine pathogenic bacterial species is summarized in Table 1. The results revealed that the essential oils showed antibacterial and antifungal activity with varying magnitudes. The zone of inhibition above 7 mm in diameter was considered as positive result. Generally, most of the tested organisms were sensitive to the essential oil tested including gram-positive and gram-negative bacteria. There was no inhibition of growth with the vehicle control (10% DMSO).

Table 1. Antibacterial activity of essential oil of *Eriosema englerianum.*

Bacteria	Negative control	*Eriosema englerianum* essential oil				Streptomycin (25 µg/disc)
		1 µl/ml	2.5 µl/ml	5 µl/ml	10 µl/ml	
Staphylococcus aureus	0	0	4.7	5.5	5.6	17.3
Escherichia coli	0	0	0	4.3	4.9	19.9
Pseudomonas aeruginosa	0	4.5	4.9	5.5	5.9	14.2
Bacillus subtilis	0	5.5	6.3	8.9	9.5	16.4
Klebsiella pneumoniae	0	0	4.4	4.6	4.7	18.9
Proteus vulgaris	0	0	4.7	6.2	6.7	18.2
Clostridium sporogens	0	4.9	6.1	6.5	7.8	15.4
Acinetobacter colcoaceticus	0	5.2	6.4	7.7	8.1	16.3

Table 2. Antifungal activity of essential oil *of Eriosema englerianum.*

Fungal species	Concentration (µl/ml)	Inhibition (%)	Poositive control (Nystatin)	Negative control
Candida albicans	1	24.2	84.3	0
	2	36.3		0
	5	42.4		0
	10	42.6		0
Aspergillus niger	1	34.7	76.3	0
	2	35.3		0
	5	42.4		0
	10	49.7		0
Aspergillus flavus	1	27.5	76.3	0
	2	34.3		0
	5	37.2		0
	10	39.3		0

The antimicrobial activity of many essential oils has been previously reviewed and classified as strong, medium or weak (Baratta et al., 1998). The results of the antibacterial tests indicated that essential oil of *E. englerianum* has medium inhibitory activity with highest activity against *B. subtilis* and *A. colcoaceticus*. Similar results were described by Graven et al. (1992). Several studies have shown that terpinolene and ascaridole had strong and consistent inhibitory effects against various pathogens (Blumenthal et al., 1998; Ali et al., 2005). Generally, gram–positive bacteria seemed to be more sensitive to the oils than gram-negative. This observation has been reported previously by other researchers (Ouattara et al., 1997; Magena and Muyima, 1999; Awouafack et al., 2008).

The essential oil was also tested against three fungal species and also showed varied activity against all the fungal organisms even though this activity was lower than that of the standard antifungal used (Table 2). The antifungal activity could be attributed to the presence of some active constituents in the oils such as O-cymene which is a natural antioxidant and animal studies suggest that an extract of O-cymene bark taken orally may help prevent stomach ulcer (Knobloch et al., 1986). This compound might be also responsible for the antioxidant activety of the essential oil from *E. englerianum*. Terpinolene, also found in this plant, has been described in other plants such as *Plectranthus cylindraceus* which also had good antimicrobial activity (Marwah et al., 2007). O-Cymene and terpinolene were both found as main components of the leaves essential oil from *Diplotaenia damavandica* which also showed strong antimicrobial activities against *B. subtilis*, *S. aureus*, *S. epidermidis* and *E. coli* (Eftekhar et al., 2005). Ascaridole was found to be a major compound of the essential oil of *Chenopodium ambrosioides* L. from Madagascar (Cavalli et al., 2004). However, the antimicrobial activity has not been determined.

An important characteristic of essential oils and their components is their hydrophobicity, which enables them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable (Gelfand et al., 1985). Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to cell death (Bhat et al., 1990). Other plants of the same genus (*Eriosema* spp.) have

been investigated and several compounds have been described with different activities. For example, two new natural dihydrochalcones were isolated from *Eriosema glomerata* including 2',4'-dihydroxy-4-methoxy-3'-(gamma, gamma-dimethylallyl) dihydrochalcone and 2',4'-dihydroxy-3'-(gamma, gamma-dimethylallyl) dihydrochalcone with significant inhibitory activity against *Bacillus megaterium*, *E. coli*, *Chlorella fusca* and *Microbotryum violaceum* (Awouafack et al., 2008). The results obtained in this study indicate the moderate antibacterial and antifungal activities of essential oils of *E. englerianum*. To our knowledge this is the first time essential oil from this plant has been investigated. Further studies are warranted to determine the activity of the oil on other organisms such as those responsible for food spoilage as well as other biological activities.

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