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Short Communication

Phytoconstituents and biological activities of essential Oil from *Rhus lancea* L. F

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The present study determined the major phytoconstituents, the antioxidant and the antimicrobial activities of *Rhus lancea* essential oil against eight bacterial and four fungal species. The yield was 0.18% and the major phytoconstituents found were ∝-pinene, benzene and δ-3-carene. The oil exhibited remarkable anti-oxidant and dose dependent antibacterial and antifungal activities with highest activities against *Escherichia coli*, *Clostridium perfringens* and *Aspergillus flavus*. To the best of our knowledge this is the first report on the antimicrobial activity of the essential oils from this plant. Further studies are warranted to test the toxicity and the suitability of this essential oil for pharmaceutical and other uses.

Key words: Antibacterial, antifungal, anti-oxidant, medicinal plant, essential oil, phytochemicals.

INTRODUCTION

Rhus lancea belongs to the family Anacardiaceae, which is the fourth largest tree family in Southern Africa of which many species are used for medicinal purposes, for food (fruits) or as building material (wood) (van Wyk and van Wyk, 1997). *R. lancea* is an evergreen, drought tolerant tree, most common in the Midlands of Zimbabwe and is also found in most areas of the Southern African region. The leaves of *R. lancea* are used as a valuable fodder for livestock and are believed to taint the flavor of milk if eaten in large quantities by diary cattle (Venter and Venter, 1996). The cause of this phenomenon is attributed to resins although there is no concrete scientific justification. In addition, there is little knowledge about its essential oils and resins, or the relationship between these products as far as the physiology of *R. lancea* is concerned.

Essential oils from plants have a great potential to substitute the synthetic raw material in food, perfume and pharmaceutical industries (Webber et al., 1999). Thus, there is a great effort to screen plants for phytoconstituents and biological activities. Some of these oils have demonstrated antibacterial, antifungal or antioxidant activity (Gundidza, 1993; Lee et al., 2003). In the present study, essential oil was prepared from fresh leaves of *R. lancea* and the chemical profile was analyzed by GC/MS. The antibacterial, antifungal and antioxidant activities of the oil were also determined; which, to the best of our knowledge, have not been studied previously.

MATERIALS AND METHODS

Plant material collection and preparation of essential oils

The plant material was collected in the district of Harare in 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. The

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Table 1. Antibacterial activity of *R. lancea* essential oil indicated by the diameter of the inhibition zone (mm).

Bacterial species	Oil concentration					
	Ethanol	10 µg/ml	20 µg/ml	50 µg/ml	100 µg/ml	Gentamicin 10 µg/ml
Acinetobacter calcoaceticus	0	7.3	8.0	13.5	14.5	18.9
Bacillus subtilis	0	7.3	7.7	9.0	12.0	16.8
Citrobacter freundii	0	7.0	8.0	10.0	11.0	17.0
Clostridium perfringens	0	5.1	6.2	11.9	15.0	16.2
Clostridium sporogenes	0	5.5	7.2	10.8	14.9	16.9
Escherichia coli	0	8.1	12.2	15.6	19.2	20.7
Klebsielia pneumoniae	0	0	0	4.1	5.3	19.0
Proteus vulgaris	0	8.3	10.6	11.2	12.0	17.8
Pseudomonas aeruginosa	0	5.2	7.6	8.3	8.5	14.5
Salmonella typhii	0	8.2	10.1	12.0	13.2	17.2
Staphylococcus aureus	0	4.0	6.2	8.1	9.5	16.1
Yersinia enterocolitica	0	6.8	9.5	9.5	9.7	17.9

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 and, after filtration, stored in dark bottles at 4°C until tested and analyzed.

Gas chromatography and mass spectroscopy analysis

Gas chromatography and mass spectroscopy analysis were conducted as previously described using a Hewlett Packard 6890 Gas Chromatograph (Adams, 2001).

Determination of the antibacterial activity of the essential oils

The antibacterial activity of the essential oil was tested using the disc diffusion methods as previously described (Gundidza et al., 1993, Samie et al., 2005). Twelve different bacterial species were used: *Acinetobacter calcoaceticus* (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). All the experiments were repeated three times and the average of three values was determined.

Determination of antifungal activity of essential Oils

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, T is the mean dry weight of the fungal cells from the test flasks. Four fungal species were used for antifungal testing namely, *Candida Albicans*, *Aspergillus Niger*, *Aspergillus flavus* and *Penicillium notatum*. All the organisms were obtained from the Department of Pharmacy, University of Zimbabwe.

Determination of antioxidant activity of essential oils

The antioxidant activity of the essential oils was determined as previously described (Gundidza et al., 1993; Burits et al., 2001). Absolute alcohol was used as a negative control and the ascorbic acids (10 mg/ml) was used as a positive control.

RESULTS AND DISCUSSION

The aim of the present study was to determine the major components of essential oils from *R. lancea* as well as the antimicrobial and antioxidant activities of the oils. The yield of the essential oil after hydrodistillation was 0.18% based on the dry weight of the plant material. Three major and two minor components were identified in the oils, representing 99.997% of the total essential oils. The three major chemical components were ∝-pinene (86.95%; retention time = 4.63 min), benzene (6.69%; retention time = 6.69 min) and δ-3-carene (20.89%; retention time = 20.89 min). The two minor components were isopropyl toluene and trans-caryophyllene of which the values were very insignificant (< 0.001%).

The essential oil showed significant antibacterial activity against all the bacterial organisms tested including all Gram (+) and all Gram (-) bacteria. The highest activity was noticed against *E. coli* (19.2 mm zone of inhibition when 100 µg/ml of oil was used compared to 20.7 mm for the positive control). The antibacterial activity may be associated with the contribution of the monoterpene α-pinene which occurred as the predominant compound in comparison to other volatile compounds (benzene, δ-3-carene, trans-caryophyllene and isopropyl toluene), and is known for antibacterial activity (Martins et al., 2000; Sökmen et al., 2003). Table 1 shows the results of the antibacterial testing indicated as zone of inhibition at different concentrations.

Essential oil of *R. lancea* displayed antifungal activity as shown in Table 2. The highest activity was observed against *A. flavus* followed by *A. niger*, while *C. albicans* appeared to be more resistant with activity less than 50% compared to 59.7% for the positive control (Clotrimazole). Although a recent review by Rayne and Mazza (2007) has indicated that there is potential for the trees members of the Rhus family, very few studies have been conducted on essential oils from this genus. Studies by

Table 2. Antifungal activity of *R. lancea* essential oil expressed as percent inhibition of the fungal growth.

Fungal species	Concentration of oil sample					
	Ethanol	2.5 µg/ml	5 µg/ml	10 µg/ml	100 µg/ml	Clotrimazole 100 µg/ml
Candida albicans	0	7.6	24.1	34.7	48.6	79.7
Aspergillus niger	0	20.2	31.6	46.9	61.9	78.2
Aspergillus flavus	0	39.4	53.7	64.4	74.2	76.2
Penicillium notatum	0	6.4	14.6	35.3	55.2	69.2

Saxena et al. (1994) have indicated that *Rhus glabra* methanolic extracts were active against several fungal species including *A. flavus, A. fumigatus, C. albicans, Fusarium tricuictum, Microsporum cookerii, M. gypseum, Saccharomyces cerevisiae, Trichoderma viridae,* and *Trichophyton mentagrophytes.* However, the essential oil from this plant was not tested.

The antimicrobial and antifungal activity is likely to be associated with the high concentration of α-pinene (86.95%). Similar findings were reported by Filipowicz et al. (2003). A synergistic effect of the benzene, δ-3-carene, isopropyl toluene and trans-caryophyllene cannot be ruled out. However, these volatile compounds occurred in minute quantities in the essential oil and there is no literature evidence, to our knowledge, that supports the existence of the antibacterial and antifungal properties in these two compounds.

The essential oil from *R. lancea* showed anti-oxidant activity by showing a mean zone of colour of 19.2 mm, which is almost the same as that produced by ascorbic acid used as positive control in the present study (20 mm). The antioxidant effect may be due to the monoterpenes α-pinene which acts as a radical scavenging agent. It seems to be a general trend that the essential oils, which contain monterpene hydrocarbons, oxygenated monoterpenes and/or sesquiterpenes have good antioxidative properties (Burits et al., 2001; Tepe et al., 2004; Mau et al., 2003).

The results obtained in the present study indicate that *R. lancea* essential oils contain medicinal properties such as antibacterial, antifungal and antioxidant activities. To the best of our knowledge this is the first report on the antimicrobial activity of the essential oils of this plant. A further research is still required to test the toxicity and the suitability of this essential oil for pharmaceutical application since *R. lancea* produces a resin which might be toxic or poisonous.

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