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Full Length Research Paper

The chemical composition and biological activities of essential oil from the fresh leaves of *Schinus terebinthifolius* from Zimbabwe

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In the present study, the essential oil from the fresh leaves of *Schinus terebinthifolius* was extracted using the hydrodistillation method. The oil yield obtained was 0.65%. Gas chromatography and mass spectroscopy (GC-MS) analysis of the essential oil showed that the major constituents of the essential oil were sabinene, α-pinene, α-phellandrene, β-pinene, terpinene-4-ol, trans-β-ocimene and myrcene. Using the hole-plate diffusion testing method, the essential oil exhibited potent antibacterial activity against *Yersinia enterocolitica, Pseudomonas aeruginosa, Escherichia coli, Acinetobacter calcoaceticus, Bacillus subtilis*, *Klebsielia pneumoniae* and *Bacillus subtilis* with at least 58% inhibition compared to the positive control. The mycelium growth inhibition method was used for anti-fungal testing. The essential oil exhibited activity against *Aspergillus flavus* with 58% inhibition*, Candida albicans* with 49.8% inhibition and *Aspergillus niger* with 48.7% inhibition. The β-carotene, acetone and linoleic acid method was used to assess the antioxidant activity of the essential oil. The essential oil showed anti-oxidant activity of 13.1 mm mean zone of colour retention representing 72.7% activity compared to the positive control (ascorbic acid). This is the first study on *S. terebinthifolius* collected from sub-saharan Africa indicating the presence of trans-β-ocimene in the essential oil and its strong antibacterial activity against *Y. enterocolitica* and *P. aeruginosa* as well as moderate antifungal activities of the plant.

Key words: Essential oil, *Schinus terebinthifolius,* Zimbabwe, antimicrobial activity*,* bacteria, fungi, antioxidant.

INTRODUCTION

Schinus terebinthifolius also known as Brazilian pepper, Aroeira, Florida holly, Rose pepper, or Christmasberry belongs to the Anacardiaceae family of plants (Manrique et al., 2008). It is indigenous to South and Central America and can also be found in semitropical and tropical regions of the United States and Africa (Ferriter, 1997). In Zimbabwe, it has been extensively cultivated for the purpose of shade and is generally found on roadsides growing on almost any type of soil. *S. terebinthifolius* is an evergreen, medium sized tree that may grow up to a height of 30 ft. It has a short trunk; intertwining branches and leaves which are pinnately compound and are dark shiny green. Leaflets have a margin almost entire to serrated and have a winged midrib. It grows well on an

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altitude of 3000 ft. Previous studies have indicated genetically differentiated source populations in the native range with contrasting genetic signatures (Pritchard et al., 2000). It is then possible that plants found in different geographic regions might have different medicinal properties based on the difference in chemical composition of the plants.

S. terebinthifolius is well known for its medicinal properties (de Lima et al., 2006). Virtually all parts of this tropical tree, including its leaves, bark, fruit, seeds, resin and oleoresin (or balsam) have been used medicinally by indigenous peoples mainly in Brazil and other South American countries but also throughout the tropics (Excell et al., 1963). The plant has a very long history of use and appears in ancient religious artifacts and on idols among some of the ancient Chilean Amerindians. In South and Central America, Brazilian peppertree is reported to have astringent, antibacterial, diuretic, digestive stimulant, tonic, antiviral and wound healing properties. In Peru for example, the sap is used as a mild laxative and a diuretic and the entire plant is used externally for fractures and as a topical antiseptic (Molina-Salinas et al., 2006). The oleoresin is used externally for wound healing, to stop bleeding and for toothaches and it is taken internally for rheumatism and as a purgative. In the Brazilian amazon, a bark tea is used as a laxative and a bark-and-leaf tea is used as a stimulant and antidepressant. In Argentina, a decoction is made with the dried leaves and is taken for menstrual disorders and is also used for respiratory and urinary tract infections and disorders (Schmourlo et al., 2005).

S. terebinthifolius has also found many medicinal applications in Africa. In South Africa, a leaf tea is used to treat colds and a leaf decoction is inhaled for colds, hypertension, depression and irregular heart beat. In Zimbabwe, a decoction from the crushed leaves or bark is traditionally taken to relieve bronchitis and rheumatism (Wild et al., 1972). Although several studies have determined the biological activities of this plant, all of them have been conducted in South America where it is native and none has been conducted in the African continent. Thus, in the present study, the essential oil was extracted from fresh leaves, collected in Zimbabwe and its antibacterial, anti-fungal as well as antioxidant activities were determined.

MATERIALS AND METHODS

Plant material

The plant material was collected in Zimbabwe with the authorization of the Zimbabwean government and in agreement with the united nation convention on biodiversity. The plant was identified by botanists at the national herbarium and botanic gardens in Harare, Zimbabwe. Three voucher specimens were deposited at the National Herbarium and Botanic Gardens in Harare, Zimbabwe and one at the herbarium of the Department of Pharmacy at the University of Zimbabwe.

Essential oil extraction

The leaves (1000 g) were subjected to hydrodistillation for approximately 3 h using a Clavenger-type apparatus. The essential oil yield obtained was 0.65% (v/w). It was dried over anhydrous sodium sulphate. After filtration, the oil was stored at approximately 4°C until tested and chemically analyzed. The essential oil was subjected to GC/MS analysis, antibacterial, anti-fungal and antioxidant testing.

Gas chromatography and mass spectroscopy analysis

A wet needle of the essential oil was inserted directly into the inlet (spitless mode) of a Hewlett packard 6890 Gas chromatograph. The temperature of the injection port was set at 220^{o}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^{o}C at 3^{o}C per min after a 3.5 min delay. Helium was also used as a carrier gas at 0.7 ml/min. Mass spectra were recorded by a HP 5937 series mass selective detector (MSD).

Antibacterial testing

The activity of the essential oil obtained from *S. terebinthifolius* was conducted using the hole plate diffusion as previously described (Gundidza, 1993). Briefly, essential oils were diluted with absolute alcohol to produce the following concentrations: 10, 20, 50 and 100% (v/v). 1 ml of the bacterial suspension was pipetted into the appropriately labeled petri dish to which 24 ml of molten agar was added. The petri dish was gently shaken to ensure thorough mixing of the contents. After the agar had set, 3 holes were punched into each plate using a vacuum agar borer. Oil (10 µl) of a specific concentration was introduced into each of the holes in an appropriately labeled petri dish using a sterile micropipette. Gentamicin (10 µg/ml) was used as a positive control and alcohol as a negative control. The dishes were then incubated at 35°C for 24 h and the zones of inhibition were measured and recorded. The inhibition zone was taken to be the diameter of the zone visibly showing absence of growth, including 4 mm hole. Where there was no inhibition, no value was assigned to the sample. 12 different standard bacterial species were used including: *Acinetobacter calcoaceticus, Bacillus subtilis, Citrobacter freundii, Clostridium perfringens, Clostridium sporogenes, Escheridia coli, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhii, Staphylococcus aureus* and *Yersinia enterocolitica.*

Determination of antifungal activity of essential oils

The mycelium growth inhibition method was used for anti-fungal testing. The medium was inoculated with 10 ml of the fungal suspension and shaken thoroughly to mix. The essential oil was then added to the flasks and for each oil concentration, 3 flasks were used. The volumes used were 10, 20, 50 and 100 µl to give the final concentrations 1, 2, 5 and 10 µl per ml respectively. In the control flasks no oil was added. The positive control had everything except the essential oil. For negative control, a nystatin pessary was crashed and dissolved in 10 ml of absolute alcohol. 100 µl of this nystatin solution was added into the positive control flasks. The flasks were incubated at room temperature and shaken occasionally for 7 days. After 7 days the fungal hyphae was collected and filtered on pre-weighed and dried filter papers. The fungi were washed three times with distilled water and then dried at 60°C overnight. The dried filter paper with hyphae was then weighed and the percentage inhibition of fungal growth found using the following

Table 1. Major chemical components of essential oil from *S. terebinthifolius* collected from Zimbabwe.

S/N	Compound	% Composition	Retention time
1	α-Pinene	30.27	4.68
2	Camphene	0.58	4.94
3	β -Myrcene	6.60	5.43
4	1-β-Pinene	7.96	5.53
5	Myrcene	1.63	5.72
6	α-Phellandrene	9.86	6.13
7	α-Terpinene	0.77	6.42
8	Sabinene	40.66	6.85
9	Trans-β-ocimene	0.30	7.18
10	γ-Terpinene	0.77	7.53
11	3-Cyclohexen-1-ol	0.61	11.45

formula: Percentage inhibition = [(C- T)/C] x 100; Where C is the mean dry weight of hyphae from the control flasks, T is the mean dry weight of hyphae from the test flasks. 4 standard fungal strains were used for antifungal testing namely, *Candida albicans, Aspergillus niger, Aspergillus flavus* and *Penicillum notatum.*

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, beta-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^{o}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.

RESULTS AND DISCUSSION

The hydrodistillation of fresh leaves of *S. terebinthifolius* collected from Harare, Zimbabwe, produced 0.65% oil (v/w). This yield is higher compared to those we have obtained from other plants such as *Rhus lancea* (0.18%) or *Eriosema englerianum* (0.28%) (Gundidza et al., 2008; Mmbengwa et al., 2009). However, this yield was far much lower compared to that obtained by Barbosa et al. (2007) from *S. terebinthifolius* fruits collected from Minas Gerais state, in Brazil. These authors described essential oil yield of 4.65% w/w after 3 h of extraction, in relation to just dry fruit weight. The differences in the oil yield could be due to the geographical difference as well as the differences that may exist in the soil types between Zimbabwe where the plant is non native and in Brazil where the plant is native. The possible difference in the climatic conditions could also explain the differences.

Gas chromatography and mass spectroscopy analysis indicated the presence of 11 major compounds that were positively identified. As indicated in Table 1 it can be noticed that the essential oil from *S. terebinthifolius* collected from Zimbabwe was mainly composed of α-pinene, sabinene, α-phellandrene, 1-β-pinene and sabinene as the major chemical components. This profile is similar to others previously described for this plant isolated from other regions of the world, particularly in Brazil. Studies by Barroso et al. (2006) indicated that β-pinene (35%), sabinene (24%) and α-pinene (21%) were the major constituents leaves of *Schinus latifolius* a close relative of *S. terebinthifolius*. In the study by Barbosa et al. (2007), about 26 compounds were identified. Similar compounds were identified with some differences in the amount of each constituent as well as the profile. For example, sabinene was found at 40.66% in our study while it represented only 3.49% in the study by Barbosa et al. (2007). Such variations between the chemical compositions of the similar plants from different geographical areas might also impact on the biological activities of the plants essential oils and extracts.

The antibacterial activity of the essential oil from this plant was tested against 12 different bacterial strains and indicated variable activities against all the organisms tested as shown in Table 2. Previous studies have indicated excellent antibacterial activities from this plant in other regions particularly in Brazil (Siddiqui et al., 1995). However, studies by de Lima et al. (2007) using extracts of this plant showed activity against *S. aureus* but, were not active against *Escherichia coli*. In the present study, the best activity was observed against *Yersinia enterocolitica* with about 95% activity when compared to the positive control. Unlike in the study by de Lima et al. (2007), the essential oil from this plant was active against *E. coli*. Strong antimicrobial activity was also observed against *P. aeruginosa* with about 77% activity when compared to the positive control. These activities may be attributed to the presence of m-cymene, 1-β-pinene, α-pinene, α-terpinene, γ-terpinene and camphene found in *S. terebinthifolius* essential oil. Enantiomers of α-pinene, 2-β-penene and limonene have been

Table 2. Antibacterial activity of *Schinus terebinthifolius* essential oil.

Bacterial species	Source	Inhibition Zone Diameter (mm)					
		Oil concentration					Positive control
		0% (v/v)	10% (v/v)	20% (v/v)	50% (v/v)	100% (v/v)	
A. calcoaceticus	NCIB 8250	0	4.7	6.2	7.35	12.0	18.9
B. subtilis	NCIB 3610	0	0	4.3	6.8	10.0	16.8
C. freundii	NCIB 11490	0	6.1	6.4	7.7	8.0	17.0
C. erfringens	ACTCC 3620	0	0	4.2	4.7	8.9	16.2
C. sporogenes	NCIB 10696	0	4.2	5.2	5.1	9.2	16.9
E. coli	NCIB 8879	0	4.3	5.1	7.2	13.2	20.7
K. pneumoniae	NCIB 4184	0	5.0	6.0	9.3	10.0	19.0
P. vulgaris	NCIB 4175	0	5.1	5.7	6.6	7.0	17.8
P. aeruginosa	NCIB 950	0	0	4.1	6.2	11.2	14.5
S. typhii	ACTCC 6539	0	4.1	5.	5.4	6.0	17.2
S. aureus	NCIB 6751	0	5.8	6.1	7.0	8.0	16.1
Y. enterocolitica	NCIB 10460	0	7.1	8.2	15.0	17.0	17.9

Table 3. Antifungal activity of *Schinus terebinthifolius* essential oil.

Fungal species	Source	Concentration of sample				Clotrimazole (100 µl/ml; positive control)
		0 µl/ml (Negative control)	2.5 µl/ml	5 µl/ml	10 µl/ml	
Candida albicans	ACTCC 10231	0	7.6	24.0	49.8	79.7
Aspergillus niger	ACTCC 10535	0	7.4	24.5	36.4	78.2
Aspergillus flavus	ACTCC 10124	0	41.2	52.2	58.1	76.2
Penicillum notatum	ACTCC 9178	0	17.9	26.1	48.7	69.2

indicated to have a strong antibacterial activity (Tzakou et al., 2001; Filipowicz et al., 2003). These chemical components exert their toxic effects against these micro-organisms through the disruption of bacteria or fungal membrane integrity (Andrews et al., 1980; Uribe et al., 1985; Knobloch et al., 1988). 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; Uribe et al., 1985). It is important to note that some of these pharmacologically active essential oil components are also present in other plants including *Melaleuca alternifolia* essential oil. Therefore it is likely that the mode of bacterial inhibition is similar to that of *M. alternifolia* oil (Cox et al., 2000). This is strongly supported by the study on the effects of different essential oil components on outer membrane permeability in gram-negative bacteria (Helander et al., 1998). The possible mechanisms of other essential oil components such as sabinene, phellandrene trans-β-ocimene and camphene have not yet been elucidated. However they may act synergistically.

The results of antifungal testing indicated moderate activities against all the 4 fungal organisms tested. From Table 3 it can be noticed that the essential oil form this plant exhibited antifungal activity against all the 4 fungal species tested. However the essential oil had less activity against *A. niger* while the highest activity was registered against *A. flavus*. The 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 phyto-constituents from the essential oil of *S. terebinthifolius* are likely to disrupt the permeability barrier of cell membranes and thereby inhibit respiration (Helander et al., 1998; Cox et al., 2000). Furthermore, it has been suggested that the differences in susceptibility of tested organisms to monoterpenes and the differences in the efficacy of different monoterpenes may possibly be explained by the variation in the rate of monoterpene penetration and characteristic membrane structure (Cox et al., 2000).

The essential oil from *S. terebinthifolius* exhibited anti-oxidant activity of 13.1 mm mean zone of colour retention compared to 18 mm for the positive control. The anti-

oxidant activity may be ascribed to the presence of the same chemical components. For example, 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). It appears that the observed radical scavenging properties of *S. terebinthifolius* essential oil might contribute positive effects in the defense of *S. terebinthifolius*. This contention is also supported by the study which showed that monoterpenes of pine and spruce namely O-cymene, 2-β-pinene, α-pinene, 1,8 – cineole, limonene, α-terpinene, α-terpinolene and camphene as well as ethanol, chalcogram and some non host green leaf alcohols [(Z)-3-hexen-1-ol, (E)-2-hexenol and 1-hexanol] appear to have inhibitory effect against attack by *Pityogenes bidentatus* bark beetle (Byers et al., 2000). Some tree species such as pines and spruce as well as *S. terebinthifolius* produce resin-like materials which contain monoterpenes to defend themselves against the penetrations of the attacking pathogens (Byers, 1995).

In conclusion our study has demonstrated differences in the chemical content of *S. terebinthifolius* growing in Zimbabwe and those growing in other part of the world particularly Brazil where the plant is native. This study has in fact highlighted the presence of chemical constituents such as 3-Cyclohexen-1-ol and trans-β-ocimene which were not observed in the oils from this plant collected in Brazil (Barboza et al., 2007). These differences might justify the differences in the antibacterial profiles observed between this study and those published from Brazil. It is possible that the antibacterial, antifungal and antioxidant activities of *S. terebinthifolius* essential oil emanated from the synergistic effect of all or some of the chemical components of the essential oil. As defense strategy against the pathogens or as an ecological adaptation to a specific habitat, many plants produce chemical compounds that act synergistically (Mau et al., 2003; Agrawal, 2007). Further evidence on the defense mechanisms has been demonstrated by the co-existance of monoterpene cyclase and oleoresin monoterpenes or by the accumulation of monoterpenes and associated volatiles after induced fungal inoculation (Lewinsohn et al., 1991; Martin et al., 2002). This study has further demonstrated the activity of the essential oil from *S. terebinthifolius,* growing in Zimbabwe, against *E. coli*, *Y. enterocolitica* and *P. aeruginosa* as well as moderate activity against *C. albicans* and *A. flavus*.

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