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Research Article

CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL OF *SCHINUS MOLLE*

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ABSTRACT

The constituents of essential oil isolated by hydrodistillation of the aerial parts of *Schinus molle* L. Anacardiaceae family, from Ethiopia was examined by GC-MS. A total of 16 components were identified accounting for 92.2 % of the oil. The essential oil of *S.molle* contained as main components α -phellandrene (20.6%), β -Phellandrene (10.8%), α -pinene (8.7%), β -pinene (5.1%), β -myrcene (6.9%), β -elemene (5.0%), copane (6.5%), germacrene (5.8%), γ -cadinene (6.3%) and α -humulene (5.4%). The essential oil of *S.molle* showed no significant antibacterial activity against gram positive (*Staphylococcus aureus*) but less inhibition towards gram negative bacteria (*Escherichia coli*).

Keywords: *Schinus molle* L , Anacardiaceae, essential oil, antibacterial activity

INTRODUCTION

Schinus molle L. (Anacardiaceae), also known as Brazilian pepper tree, is a tree which is short and has thin, long leaves and it is often used in subtropical climates for landscaping. *Schinus* species has been traditionally used as medicine by indigenous people throughout the tropics. Pharmacological studies conducted with extracts from *Schinus molle* showed that this plant has hypertensive, antitumor, antifungal, antibacterial, anti-inflammatory, analgesic, and antidepressant properties¹⁻¹⁰. It also exhibited antifungal activity towards storage fungi and insect repelling activities¹¹⁻¹³.

MATERIALS AND METHODS

Plant material

The aerial parts of *Schinus molle* plant was collected during the month of January 2012 from Mekelle, Ethiopia. The plant was identified by the authors and its herbarium sheet was deposited at the post graduate department of Chemistry, Mekelle University.

Essential oil extraction

The shade dried aerial parts of *Schinus molle* plant collected (1Kg) was subjected to hydro distillation in a Clevenger apparatus for 3h. The essential oil was separated from aqueous layer using a 100 mL capacity separator funnel. The collected essential oil was dried over anhydrous sodium sulphate and filtered using a Whatman filter paper no. 40. The extracted essential oil was colorless liquid in appearance which was stored at 4°C in dark brown 5-mL capacity sample bottle until

analysis. The yield of the oil was found to be 2.0% (w/w) in relation to the dry weight basis.

GC and GC-MS analysis

GC analyses were carried out in Agilent Technology 6890N gas Chromatograph data handling system equipped with a split-split less injector and fitted with a FID using N₂ as carrier gas. The column was HP-5capillary column (30m x 0.32mm,0.25 μ m film thickness)and temperature program was used as follows: initial temperature of 60 °C(hold : 2 min) programmed at a rate of 3⁰C/min to a final temperature of 220⁰C(hold : 5 min).Both the temperature of injector and FID were maintained at 210⁰C.

The GC-MS was performed by Perkin Elmer Clarus 500 gas chromatograph equipped with a split-split less injector (split ratio 50:1) data handling system. The column was an Rtx®-5 capillary columns (60 min x 0.32 mm, 0.25 μ m film thickness).Helium was used as carrier gas at a flow rate of 1.0ml/min.The GC was interfaced with Perkin Elmer 500 mass detector operating in EI⁺ mode.The mass spectra was recorded over 40-500 amu and revealed the Total Ion Current chromatograms.The temperature program remained the same as in GC.The temperatures of injector and transfer line were kept at 210⁰ C and that of ion source at 200⁰C.

Identification of the oil components was done by comparison of their mass spectra with the NIST/Wiley library as well as by comparing them with those reported in literature. The identification of each compound was also confirmed by comparison of its retention index with those of authentic compounds¹⁴.

Antibacterial activity

The study was conducted by using standard disc diffusion method. In each experiment, microorganisms were cultured at 37 °C for 24 hours and prepared to turbidity which is equivalent to 0.5 McFarland standards (National Committee of Clinical Laboratory Standards)¹⁵⁻¹⁷.

Mueller-Hinton (MH) agar 38g was dissolved in 1000 ml of distilled water. Then it was boiled on heating mantle to dissolve the media completely and then sterilized by autoclaving at 15 lbs. and 121°C for 15 min. After it was autoclaved at indicated conditions, it was poured to the sterilized petridishes and allowed to set at room temperature until the agar has solidified. It was then incubated at 37 °C for 24 hours to be ready for susceptibility test.

The stock solution of the crude *Schinus molle* oil in Chloroform (20mg/ml) and test discs were prepared from Whatman filter paper.

A 0.5 McFarland standard was prepared as described in National Committee of Clinical Laboratory Standards (NCCLS)¹⁸⁻¹⁹. One percent V/V solution of sulfuric acid and 1.175% W/V solution of barium chloride were prepared and made it turbidity standard. A small volume of this turbid solution was transferred to a screw capped tube and vigorously shaken on a mechanical vortex mixer to have a uniform turbid appearance and stored in the dark at room temperature.

Purely cultured Mueller-Hinton agar petridishes were labeled with different names of bacteria. Then 5 ml of sterile Normal Saline Solution (NSS) was pipetted out into a three different sterile screw-cap tubes. These tubes were labeled according to the type and number of bacteria used to test (*E. coli* and *S. aureus*). To prepare inoculums, 3 well isolated colonies of the same morphological types were selected from an agar plate culture. The top of each colony is touched with a loop, and growth was transferred into a tube containing 5 ml of NSS that corresponds to each bacterium names. These inoculums containing tubes were mixed by using mechanical vortex mixer and their turbidity was compared accurately.

The sterile discs which were prepared by office perforator were inserted in to different concentrations of *Schinus molle* oil with stock solution of 20mg/ml. It was impregnated in to negative and positive controls petroleum ether and chloroform, and amoxicillin respectively. After that, discs with different concentrations were placed on the inoculated plates using a pair of sterile forceps. Seven discs were placed on a 90 cm diameter petridish plate and the space between each disc was given as 24 mm gap from center of the disk to the center of petridish. The pressed discs were completely stacked the agar surface, plates were inverted and placed in an incubator at 37 C for 24 hour. After overnight incubation, the diameter of each zone (including the diameter of the disc) were measured and recorded.

Table 1: Chemical composition of essential oil of *S. molle*

Peak No	RI	Compounds Identified	% Composition
1	986	α - Pinene	8.7
2	1011	trans-Piperitol	3.5
3	1024	α -Phellandrene	20.6
4	1048	β -Pinene	5.1
5	1030	β -Phellandrene	10.8
6	1130	β -Myrcene	6.9
7	1140	β -Elemene	5.1
8	1390	Copane	6.5
9	1423	Isolidene	1.7
10	1428	Germacrene	5.8
11	1448	α - Cubebene	1.4
12	1480	Aristolene	1.8
13	1520	δ -Cadinene	6.3
14	1560	α -Humulene	5.4
15	1620	α -Gurjunene	1.3
16	1640	β -Caryophyllene	1.4

Table 2 *In vitro* antibacterial test of *Schinus molle* oil

Test bacterial strains	Inhibition zone in (mm)							
	Concentration of crude oil (mg/ml)				Number of fractions		Positive control	Negative control
	20	10	5	1	17	18	amoxicillin	chloroform
<i>Escherichia coli</i>	7.5±0.4	-	-	-	7±0	7±0	20±0	-
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	20±0	-

Key: All values are given as mean \pm SDV which were done in doublets.

RESULTS AND DISCUSSION

The composition of essential oil of *Schinus molle* is shown in the table 1. A total of 92.2% was identified. The major components identified were α -phellandrene (20.6%), β -

phellandrene (10.8%), α -pinene (8.7%), β -pinene (5.1%), β -myrcene (6.9%), β -elemene (5.0%), copane (6.5%), germacrene (5.8%), γ -cadinene (6.3%) and α -humulene (5.4%). The above identified constituents in the present study were reported by earlier workers²⁰⁻²³. A report from Italy on

the essential oil constituents of *Schinus molle* showed to contain α -phellandrene, limonene, β -phellandrene, myrcene, elemol, camphene, sabinene, α and β -pinene as the main compounds in the leaf oil²⁴. An investigation from Brazil showed the presence of sabinene and limonene as the main constituents²⁵. A recent report from Saudi Arabia showed that the leaf oil of *S. molle* contained, *p*-cymene, α -terpinene and β -pinene as the main volatile constituents²⁶.

The essential oil of *Schinus molle* showed moderate antibacterial activity (table 2) against gram negative (*E. coli*) bacteria while no zone of inhibition towards gram positive bacteria (*S. aureus*).

This report is totally in agreement with an earlier investigation²⁷. This antibacterial activity is attributed to the presence of active principles such as phellandrenes, myrcene and pinene in the oil of *S. molle*^{21, 22, 26}.

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