Research Journal of Applied Sciences 1 (1-4): 132-135, 2006

Chromatographic Analysis, Antimicrobial and Antifungal Activities of Essential Oil Constituents Obtained from *Vitellaria paradoxa*

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Abstract: A modified Clevenger hyrodistillation apparatus was used to obtain essential oils from the leaf, stem bark and root of Vitellaria Paradoxa plant. Thin Layer Chromatographic (TLC) analysis of the oils in 1:1 and 3:1 hexane-ethyl acetate solvent mixtures gave Retention Index (RI) values indicative to the likely presence of geraniol, β-pinene, D-carvone, L-carvone, Eugenol and citral. Antimicrobial and antifungal screening of the oils using a good number of pathogenic microorganism at 50-300 mg mL^{-1} concentrations indicated high activity on a large number of bacteria and fungi.

Key words: Vitellaria paradoxa, volatile essential oils, geraniol, β-pinene, D-carvone, L-carvone, eugenol, citral, *E-coli*

INTRODUCTION

Essential oils are odoriferous complex mixture of volatile substances in living organism. They are oily products of plant or animal origin, which are distillable and non-miscible but could be slightly soluble in water[1].

These oils are produced in the protoplasm of plant cells and are products of metabolism and are also stored as micro-droplets in glands of the plant[1] .

Vitellaria paradoxa belongs to the kingdom plantae, phylum spermatophyta, subphylum angiosperm, class dicotyledon, order ebenales and family sapotaceae[2,3]. The tree is very polymorphic, resistant to bush fire and is very long lived, perhaps up to 300 years[3]. The early Arab traveler Ibn Batouta recorded them in 1356 that the fruit is an important source of oil for many usages in cooking, illumination of lamps, ointments and even as a building substance mixed with soil to produce a hard mortar[4,5].

The bark decoction of the plant is administered in upper volta in draught or bath to promote child birth during final labour[6]. It is used as medicine for stomach ache by salka people of Northeast Nigeria while the kimba's take it for diarrhoea[3,6]. The stem-bark is used for skin infections remedy while the root is used in Nigeria as chewing stick. Ghanaians grind the root to powder as is taken with porridge for fever with Jaundice[7].

The extensive medicinal use of this plants encouraged us to isolate the essential volatile oils from its leaf, stem-bark and root in an attempt to characterize the chemical constituents chromatographically, scientifically evaluate the antimicrobial and antifungal activities to support the medicinal importance of this plant.

MATERIALS AND METHODS

Sampling and preparations of plant materials: Mature vitellaria paradoxa whole plant was collected from a location around Ibadan, Nigeria identified and authenticated by Mr. T.K Odewo of the Herbarium section, Forestry Research Institute of Nigeria (FRIN).

The fresh whole plant sample were air dried for about 5-7 days and subsequently pulverized separately into leaf, stem-bark and root using a grinding machine to increase the surface area for extraction of oils from the oil bearing glands.

ISOLATION OF ESSENTIAL OILS

500 g each of the pulverized sample were separately and carefully introduced into a 5 litre flask and water added until the sample is well immersed. Hydrodistillation was then carried out for about $3^1/_2$ h in an all glass Clevenger distillation apparatus designed according to BP specifications[8].

The volatile oil was trapped in about 1.5 mL hexane in the receiver arm of the Clevenger apparatus. The weight

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of the extracted oil per sample weight was determined and the volume obtained measured.

TLC ANALYSIS OF THE ESSENTIAL OILS

Thin Layer Chromatographic (TLC) analysis of the essential oils was done in two solvent systems comprising of a mixture of 1:1 and 3:1 hexane-ethyl acaetate solvent ratios. The Retention Index (RI) value was calculated with the relationship:

$$RI = \frac{\text{Distance moved by component of sample}}{\text{Distance moved by solvent}}$$

ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OILS

Agar well-diffusion method: Sterile assay media (Mueller-Hinton agar and antibiotic sensitivity agar) were seeded with the indicator organisms by streaking the entire surface of the culture plates and incubating at appropriate temperatures for 3 h. Holes 6 mm in diameter were aseptically punched out of the agar plates and then, the essential oils were separately dispensed into the holes followed by incubation at 37°C for 24-48 h. The demonstration of antagonism depends on the release into the assay media of diffusible inhibitors (essential oils) early in the growth phase of the producer organisms.

MIC determination: Inhibitory zones surrounding the indicator organism s were noted and recorded in mm diameter.

The minimum inhibitory concentrations (MIC) of the essential oils were determined using the dilutions susceptibility method. A series of dilutions were made from the concentrated essential oils in the ratio 1:9. About 0.5 mL of each of the diluents were separately dispensed into the agar wells after surface streaking of the sterile assay plates (Mueller-Hinton agar and Antibiotic sensitivity agar) by the indicator organisms. This was followed by incubating the plates at appropriate temperatures of 37°C for 24-48 h. Inhibitory zones surrounding the indicator organisms were also noted and recorded in mm diameter. The lowest concentrations of the essential oils resulting in no growth of the indicator organisms after 24-48 h is the MIC.

Concentration of essential oils used

- Vitellaria paradoxa (leaf) 50 mg mL^{-1}
- Vitellaria paradoxa (stem bark) 150mg mL^{-1}
- Vitellaria paradoxa (root) 60 mg mL^{-1}

RESULTS AND DISCUSSION

The percentage yield, colour and odour of the essential oils obtained from the leaf, stem bark and root of the plant is presented in Table 1.

Table 1 reveals that the percentage yield of the essential oils obtained are very low. This may be adduced to factors such as period of collection of the plant samples, age of plant and loss due to vapourisation during hydrodistillation.

The Retention Index (RI) values determined with reference to known standard constituent of essential volatile oils compared with some components of the essential oils of the samples under study are shown in Tables 2 and 3. The tables show that the two solvent systems used produced good separation of the essential oils which on comparison of their RI values with the known standard components indicated the likely presence of Geraniol α, β-Pinene, L-carvone, Eugenol β-ionone and citral in the leaf, Geranol, L-carvone and β-pinene in stem-bark while Geranol, β-pinene, Eugenol and L-carvone, are the likely constituents of the root samples.

Table 1: Colour, odour and percentage yield of essential oils

Source of essential oil	Colour	Odour	%yield (W/W)
Vitellaria paradoxa (leaf)	Pale yellow	Pungent	0.1
Vitellaria paradoxa (stem-bark)	Pale yellow	Pungent	0.2
Vitellaria paradoxa (root)	Colourless	Pungent	0.1

Table 2: TLC results in 1:1 hexane-ethylacetate solvent system

	Retention index	Retention index (samples)		
Standards	(standards)	Leaves	Stem bark	Roots
Citral	0.76	-	-	-
D-carvone	0.73	-	-	-
Iso-safrole	0.73	-	-	-
α-Pinene	0.73	-	-	-
β-Pinene	0.63	0.62	-	-
Eugenol	0.74	-	-	0.74
L-carvone	0.86	0.82	0.85	0.82
Guaiazulene	0.93	-	-	-
Geraniol	0.54	0.54	0.57	0.58
β-Pulegone	0.71	-	-	-

Table 3: TLC results in 3:1 hexane-ethylacetate solvent system

		Retention index (samples)		
Standards	RI (standards)	Leaves	Stem bark	Roots
Citral	0.56	0.57	-	-
D-carvone	0.49	-	-	-
α-ionone	0.54	-	-	-
α-pinene	0.26	0.24	-	-
β-Pinene	0.31	-	0.29	-
Eugenol	0.39	0.42	-	-
L-carvone	0.58	-	-	-
Guaiazulene	0.65	-	-	-
Geraniol	0.35	-	-	-
Citronellol	0.37	-	-	-
β-ionone	0.52	0.50	-	-
No known standard	> 0.65	0.81	-	-
No known standard	< 0.26	-	-	0.15

Table 4: Antimicrobial effect of essential oils on bacterial Isolates after 48 h

Bacterial isolates	Code	Isolate source	Essential oil source	Zone of inhibition (mm)	Susceptibility
Salmonella enterica serovar Typhii	V3^{6}9b^{2}	Vomit flora	*V.paradoxa (root)*	30	High
Klebsiella pneumoniae	CD50(3)	Faecal flora	*V. paradoxa (leaf)*	15	Low
Klebsiella aerogenes	CD 506 (5)	Faecal flora	*V. paradoxa (leaf)*	20	Moderate
Escherichia coli	CD 2295)	Faecal flora	*V. paradoxa (leaf)*	15	Low
Shigella dysentariae	OR 38 (4)	Oral flora	*V. paradoxa (root)*	25	Moderate
Pseudomonas aeruginosa	CD 50b (5)	Faecal flora	*V. paradoxa (leaf)*	25	Moderate
Pseudomonas aeruginosa	CD 50b (6)	Faecal flora	*V. paradoxa (stem bark)*	20	Moderate
Pseudomonas aeruginosa	CD 50b (7)	Faecal flora	*V. paradoxa (root)*	25	Moderate
Pseudomonas aeruginosa	CD 5D (3)	Faecal flora	*V. paradoxa (leaf)*	27	Moderate
Pseudomonas aeruginosa	CD 5D (4)	Faecal flora	*V. paradoxa (root)*	15	Low
Staphylococcus aureus	CD 22X (5)	Faecal flora	*V. paradoxa (stem bark)*	16	Low
Staphylococcus aureus	CD 22X (6)	Faecal flora	*V. paradoxa (root)*	12	Low
Staphylococcus aureus	CD 22^{2} (4)	Faecal flora	*V. paradoxa (leaf)*	20	Moderate
Bacillus licheniformis	V392^{2} (5)	Vomit flora	*V. paradoxa (leaf)*	20	Moderate
Bacillus licheniformis	V392^{2} (6)	Vomit flora	*V. paradoxa (stem bark)*	18	Low
Bacillus licheniformis	V392^{2} (7)	Faecal flora	*V. paradoxa (root)*	20	Moderate
Proteus sp	CD 38^{10} (5)	Faecal flora	*V. paradoxa (leaf)*	13	Low
Proteus sp	CD 43 (2)	Faecal flora	*V. paradoxa (leaf)*	22	Moderate
Proteus sp	CD 43 (3)	Faecal flora	*V. paradoxa (root)*	25	Moderate
Klebsiella sp	CD 44^{2}1 (4)	Faecal flora	*V. paradoxa (stem bark)*	20	Moderate
Klebsiella sp	CD 49 (5)	Faecal flora	*V. paradoxa (leaf)*	15	Low
Klebsiella sp	CD 50^{2} (3)	Faecal flora	*V. paradoxa (stem bark)*	30	High

Table 5: Antimicrobial effect of essential oils on fungal isolates after 48 h

Fungal isolates	Code	Essential oil source	Zone of Inhibition (mm)	Comment
Candida tropicalis	C. 5	*V. paradoxa (stem bark)*	30	High
Candida tropicalis	C. 5	*V. paradoxa (root)*	25	Moderate
Candida glabrata	C. 53	*V. paradoxa (leaf)*	12	Low
Candida glabrata	C. 53	*V. paradoxa (stem bark)*	25	Moderate
Candida glabrata	C. 53	*V. paradoxa (root)*	25	Moderate
Candida glabrata	C. 92	*V. paradoxa (root)*	20	Moderate

Table 6: MIC of essential oils on selected bacterial and fungal isolates

			Zones of inhibition (concentration)			
Isolate	Code	Essential oil source	$X10^{0}$	$X10^{-1}$	$X10^{-2}$	$X10^{-4}$
Proteus (Sp) (Bacterial)	CD 43	V. Paradoxa (leaf)	20	-	-	-
Pseudomonas aerogeneses (Bacterial)	CD 5D	V. Paradoxa (leaf)	30	-	-	-
C.glabrata (Fungal)	C53	V. Paradoxa (stem bark)	30	-	-	-
C. glabrata (Fungal)	C. 53	V. Paradoxa (root)	30	22	-	-

Eight strains of shigella dystenteriae OR7 (4) and OR 38 (4) isolated from gastro enteritic clinical specimens (stools and vomitus) were screened against the essential oils. Results shown in Table 4 reveals a low (10.0mm in diameter) susceptibility to V. paradoxa root but resistant to essential oils from the leaf and stem bark.

Eleven strains of Pseudomonas aeruginosa isolated from faecal specimens were screened against the essential oils and all were moderate (20.0-25.0 mm in diameter) in susceptibility except pseudomonas aeruginosa CD5D (4) that had low susceptibility (15.0 mm in diameter).

Twenty seven strains of klebsiella, klebsiella aerogenes (6), klebsiella pneumoniae (8) and klebsiella sp (13) were screened against the essential oils and they all exhibited between low (13.0 mm in diameter) and high (30 mm in diameter) susceptibility Table 4.

Eight proteus sp and one proteus mirabilis were screened and they all displayed between low and moderate susceptibility Table 4. Of the ten staph aureus strains screened, seven were low in susceptibility while the remaining three were moderately susceptible to the essential oils. Seven B.licheniformis isolated from vomitus specimens of gastroenteritic patients were screened against the essential oils and all exhibited moderate susceptibility except B. licheniformis V392 (6) that exhibited low susceptibility Table 4.

Out of a total of thirty-two candida strains implicated in sexually transmitted infections, (C. albicans (3), C tropicalis (13), C. glabrata (15), C. pseudotropicalis (1) which was screened against the essential oils, Four of the C. tropicalis were low (15.0-18.0 mm in diameter); six were moderate (20.0-25.0 mm in diameter) while three were high

(30.0-35.0 mm in diameter) as regards their susceptibility values. Among the C. glabrata strains two had low (12.0-15.0 mm in diameter); eight had moderate (20.0-25.0 mm in diameter) and five had high (30.0-35.0 mm in diameter) susceptibility towards the essential oils Table 5. Out of the three C. albicans strains one was low while the other two were moderate in their susceptibility towards the essential oils Table 5.

The minimum inhibitory concentrations of the essential oils on selected bacterial and fungal isolates based on the activity results shows that the oils were quite active even at low concentrations Table 6.

CONCLUSION

Vitellaria paradoxa whole plant has shown from this study to possess good antimicrobial and antifungal activities and thus the various traditional uses could be justified. The plant which is already in use as spices could be very useful if its chemotherapeutic properties could be fully exploited.

The Thin Layer Chromatographic (TLC) analysis gave results suggesting which of the suspected components is responsible for the antimicrobial and antifungal activities of the oil. However, a GC/MS analysis will need to be done to further identify the responsible components.

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