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In vitro antibacterial activity and gas chromatography–mass spectroscopy analysis of *Acacia karoo* and *Ziziphus mauritiana* extracts

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Abstract

The aim of the study was to identify the chemical ingredients and to evaluate the antibacterial activity of crude extracts of locally grown *Acacia karoo* and *Ziziphus mauritiana*. Antibacterial activity was measured by the agar well diffusion method, and chemical ingredients were identified by gas chromatography–mass spectrometry. Qualitative analysis of crude organic extracts confirmed the presence of high- and low-molecular-mass compounds. The extracts had a broad spectrum of antibacterial activity. An ethyl acetate extract of *A. karoo* root induced a maximum zone of inhibition of *Staphylococcus aureus* (35 ± 1.15 mm) and the least activity against *Proteus vulgaris* (10 ± 1.52 mm). A methanol extract of *Z. mauritiana* root induced a maximum zone of inhibition of *Escherichia coli* (35 ± 1.15 mm) and had the least activity against *Klebsiella pneumoniae* (10 ± 1.52 mm). We conclude that root extracts of *A. karoo* and *Z. mauritiana* have significant antibacterial activity.

Keywords: *Acacia karoo*; *Ziziphus mauritiana*; Antibacterial activity; Gas chromatography–mass spectrometry

1. Introduction

Microbial infections are major health hazards and are the cause of nearly 25% of all deaths, according to the World Health Organization [1]. Microbial drug resistance has recently been documented around the world [2,3], with the emergence of new infectious diseases; therefore, the development of new, effective antimicrobial drugs is vital. As the current synthetic drugs are toxic and cause many side-effects, plants have received much scientific attention for the development of alternative drugs to cure several lethal diseases [4–6]. Plants and herbs have been used as a source of therapeutic compounds since ancient times in traditional medicinal systems. Indian systems of medicine like *ayurveda*, *unani*, *siddha*, *yoga* and naturopathy are some of the oldest in the world. These systems include use mainly of plants or plant products. Although in traditional treatment, crude extracts were used, it is now essential to identify and characterize new phytochemical

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components of plants. The antimicrobial activities of locally grown medicinal plants have already been the subject of a number of studies [7–9].

Acacia karoo is tree native to India, Sri Lanka and the USA. It is commonly known as *kikar*. The tree grows to a height of 12 m with a trunk diameter of 1.2 m. Its leaves are deciduous, bi-pinnate, light green, with 12–120 leaflets. Its roots grow to 53 m in depth. The plant has an unusually large amount of flavonol-mesquitol in its heartwood, and the leaves, roots, stems and latex of *A. karoo* are used in traditional medicine against several diseases [10].

Ziziphus mauritiana is a plant native to the Indo-Malaysian region of South-East Asia, southern Africa, China, Australasia and the Pacific Islands. Its fruit is commonly known as *ber* or Indian plum. *Z. mauritiana* is a spiny, evergreen shrub and small tree with a spreading crown, stipular spines and many drooping branches. Its fruit is nutritious and rich in vitamin C. It contains 20–30% sugar, up to 2.5% protein and 12.8% carbohydrates. The leaves are readily eaten by camels, cattle and goats. The root is purgative, and a root decoction is given as a febrifuge, taenicide and emmenagogue; powdered root is dusted onto wounds. The juice of the root bark is said to alleviate gout and rheumatism, although strong doses of the bark may be toxic [11].

We studied the antibacterial activity and composition of *A. karoo* and *Z. mauritiana* extracts.

2. Materials and methods

2.1. Chemicals and reagents

Nutrient agar, nutrient broth, Mueller-Hinton agar and ampicillin were purchased from Himedia Pvt Ltd, Mumbai, India. Methanol, ethanol, chloroform and ethyl acetate were purchased from SRL Pvt Ltd, Mumbai, India.

2.2. Plant materials

Leaves and roots of *A. karoo* were collected from the desert in Rajasthan (26.5727° N, 73.8390° E) and leaves and roots of *Z. mauritiana* from the hills of Jhunjhunu (28.1300° N, 75.4000° E), Rajasthan, in September 2013. The plant materials were taken to the Biosciences Laboratory, JJT University, Jhunjhunu and authenticated by G.M. Kachhawa, a botanist, at Shri JJT University. A voucher specimen of each plant was maintained in our laboratory for future reference.

The plant material was washed thoroughly in tap water followed by distilled water, then shade-dried at room temperature. Dried leaves and roots were ground uniformly in a mechanical grinder, and the pulverized plant materials were serially extracted with chloroform, methanol, ethanol and ethyl acetate. The extract was concentrated in a rotary evaporator, and dried extract was kept in airtight containers at 4 °C until further use.

2.3. Antibacterial assay

The extracts were tested against the clinically pathogenic bacteria *Staphylooccus aureus*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Bacillus subtilis*. Ampicillin and distilled water were used as positive and negative controls, respectively.

The antibacterial activity of the extracts was determined by the agar well diffusion method as described by Gaurav et al. [20]. The concentration of bacteria suspension was adjusted to 0.5 McFarland standards and seeded onto Mueller-Hinton agar plates. Three wells

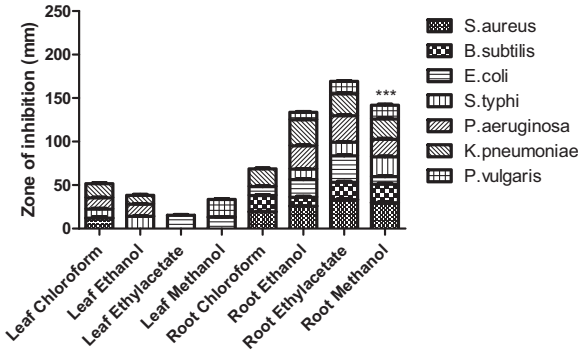

Fig. 1. Antibacterial activity of *A. karoo*.

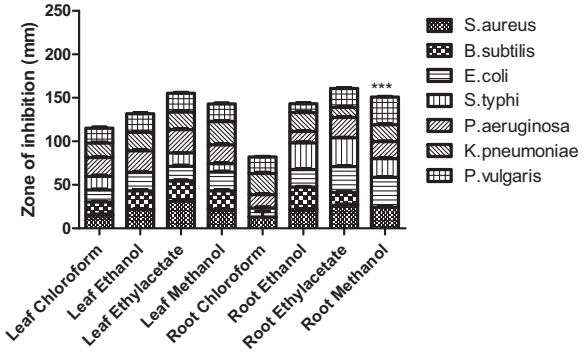

Fig. 2. Antibacterial activity of *Z. mauritiana*.

Table 1
Chemical ingredients of different extracts of *Acacia karoo*.

Extracts	Retention time	Compound name	M.W.	Formula	CAS
Leaf chloroform	20.077	Cyclohexanone, 2-methylene-5-(1-methylethyl)-	152	C10H16O	15297-07-1
	24.112	Cyclohexanone, 2-methylene-5-(1-methylethyl)-	152	C10H16O	15297-07-1
	24.242	Cyclohexanone, 2-methylene-5-(1-methylethyl)-	152	C10H16O	15297-07-1
	24.487	Cyclohexanone, 2-methylene-5-(1-methylethyl)-	152	C10H16O	15297-07-1
	25.117	399 Cyclohexanone, 2-methylene-5-(1-methylethyl)-	152	C10H16O	15297-07-1
	26.398	4-Methyl-2,4-bis(4′-trimethylsilyloxyphenyl)pentene-	412	C17H30OS	900283-56-8
	27.273	Trimethyl[4-(1,1,3,3,-tetramethylbutyl)phenoxy]silane	278	C17H30OSi	78721-87-6
	27.823	Trimethyl[4-(1,1,3,3,-tetramethylbutyl)phenoxy]silane	378	C17H30OSi	78721-87-6
Leaf ethyl acetate	10.772	Cyclotrisiloxane, hexamethyl-	222	C6H18O3Si3	541-05-9
	12.908	Cyclotrisiloxane, hexamethyl	222	C6H18O3Si3	541-05-9
	14.463	Methyl-2,4-bis(4′-trimethylsilyloxyphenyl)pentene-	412	C24H36O2Si2	900283-56-8
	25.112	Cyclohexane, 1-methyl-4-(1-methylethenyl)-, trans-	138	C10H18	1124-25-0
	26.473	Furan, 2-hexyl-	152	C10H16O	3777-70-6
	26.728	-, (Bicyclo[2.2.1]heptan-2-one, 4,7,7-trimethyl 1s	152	C10H16O	10292-98-5
	27.483	Trans-decalin, 2-methyl-	152	C11H20	900152-47-3
	29.494	Cyclotrisiloxane, hexamethyl	222	C12H22Si2	541-05-9
Leaf ethanol	16.89	Trimethyl[4-(1,1,3,3,-tetramethylbutyl)phenoxy]silane	278	C6H18O3Si3	78721-87-6
	17.15	Cyclotrisiloxane, hexamethyl	222	C6H18O3Si3	541-05-9
	17.35	Methyl-2,4-bis(4′-trimethylsilyloxyphenyl)pentene-	222	C24H36O2Si	541-05-9
	19.6	Oxamide, N-[3-(1-imidazolyl)propyl]-N′-methyl-	210	C9H14O2N4	900277-31-6
	28.739	Cyclodecene, 1-methyl-	152	C11H20	66633-38-3
	29.779	Acetamide, N-(3-imidazol-1-ylpropyl)-2-methoxy-	197	C9H15O2N3	900303-03-4
	30.835	Cyclohexene, 1-pentyl-	152	C11H20	15232-85-6
	31.515	Bicyclo[2.2.1]heptan-2-one, 4,7,7-trimethyl-, (1r)-	152	C10H16O	13854-85-8
Root chloroform	16.829	Bicyclo[2.2.1]heptan-2-one, 4,7,7-trimethyl-, (1s)-	152	C10H16O	10292-98-5
	24.077	3-(4,8,12-Trimethyltridecyl) furan	292	C20H36O	900245-55-1
	24.112	Trans-decalin, 2-methyl-	152	C11H20	900152-47-3
	24.242	Cis-decalin, 2-syn-methyl	152	C11H20	900155-85-6
	24.487	Bicyclo[4.1.0]heptan-3-one, 4,7,7-trimethyl-, [1r-(1.alpha.,4.alpha.,6.alph	152	C10H16O	900155-85-6
	25.117	1,2-Bis(trimethylsilyl)benzene	222	C13H22OSi	17151-09-6
	26.398	Trimethyl(4-tert.-butylphenoxy)silane	222	C12H22Si2	25237-79-0
	27.273	Methyl-2,4-Bis(4′-trimethylsilyloxyphenyl)pentene-1	222	C6H18O3Si3	25237-79-0
Root ethyl acetate	27.454	Bicyclo[4.1.0]heptan-3-one, 4,7,7-trimethyl-, [1r-(1.alpha.,4.beta.,6.alpha	152	C10H16O	4176-01-6
	20.296	Furan, 2-hexyl-	152	C10H16O	3777-70-6
	21.961	Spiro[5.5]Undecane	152	C11H20	180-43-8
	23.532	Cyclodecene, 1-methyl-	152	C11H20	66633-38-3
	24.312	Cis-decalin, 2-syn-methyl-	152	C11H20	900155-85-6
	26.373	Cyclotrisiloxane, hexamethyl	222	C12H22Si2	541-05-9
	27.118	Cyclotrisiloxane, hexamethyl	222	C6H18O3Si3	541-05-9
	28.799	Cyclotrisiloxane, hexamethyl	222	C15H24O2Si	541-05-9
Root ethanol	18.24	Bicyclo[4.1.0]heptan-3-one, 4,7,7-trimethyl-, [1r-(1.alpha.,4.beta.,6.alpha.	152	C10H16O	4176-06-1
	19.51	2-Cyclohexen-1-one, 2-methyl-5-(1-methylethyl)-, (S)-	152	C10H16O	499-71-8
	25.107	Bicyclo[4.1.0]heptan-3-one, 4,7,7-trimethyl-, [1r-(1.alpha.,4.beta.,6.alpha	152	C10H16O	2547-27-5
	26.448	1,2-Benzisothiazol-3-amine Tbdms	222	C12H22Si2	900332-57-2
	27.473	Trimethyl(4-tert.-butylphenoxy)silane	222	C13H22OSi	25237-79-0
	29.449	Cyclotrisiloxane, hexamethyl	222	C6H18O3Si3	541-05-9
	29.464	Bicyclo[4.1.0]heptan-3-one, 4,7,7-trimethyl-, [1r-(1.alpha.,4.alpha.,6.alph	152	C10H16O	4176-06-1
	30.555	Cyclodecene, 1-methyl-	152	C11H20	66633-38-3

were cut with a standard cork borer (7 mm), and 100 μl of each extract and controls were added to the wells. After incubation for 24 h at 37 °C, antibacterial activity was evaluated by measuring the zone of inhibition. Experiments were performed in triplicate.

2.4. Gas chromatography–mass spectrometry (GC–MS)

GC-MS analyses of organic crude extracts of the leaves and roots of *A. karoo* were performed in a Perkin

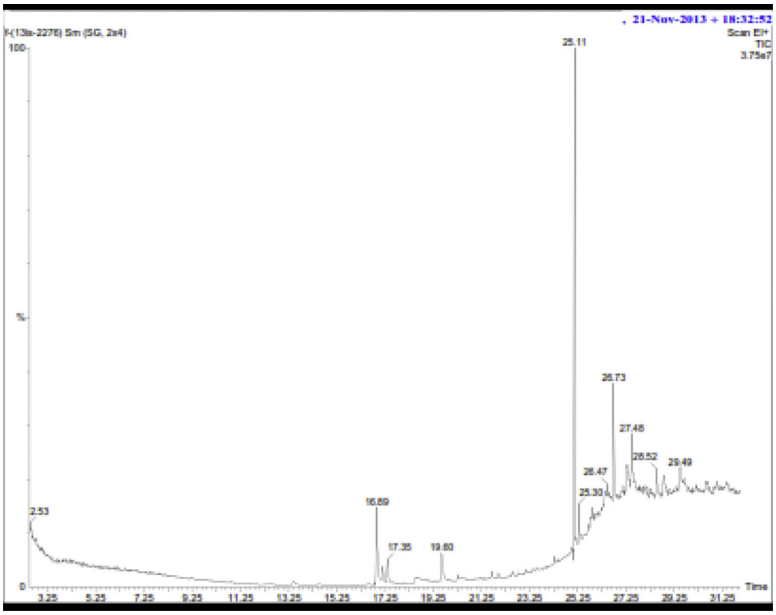

Fig. 3. Gas chromatogram of chemical ingredients of chloroform extract of *A. karoo* leaf.

Fig. 4. Gas chromatogram of chemical ingredients of ethanol extract of *A. karoo* leaf.

Elmer model Clarus 680 equipped with a mass spectrometer EI (Clarus 600) on an Elite-5MS (30.0 m, 0.25 mm ID, 250 μm df) column. The initial oven temperature was 60 °C for 2 min, ramped at 10 °C/min to 300 °C and held for 6 min. Helium gas as the carrier gas was at a constant flow rate of 1 mL/min. The mass transfer line and source temperature were both set at 230 °C. Chemical constituents of *A. karoo* were identified by matching their recorded mass spectra with those obtained from the Wiley8.LIB and NIST08.LIB library spectrum.

2.5. Statistical analysis

The data were expressed as means ± standard deviation of triplicate analyses and analysed by two-way ANOVA. $p < 0.05$ was considered statistically significant. Prism software was used for data analysis.

Fig. 5. Gas chromatogram of chemical ingredients of ethyl acetate extract of *A. karoo* leaf.

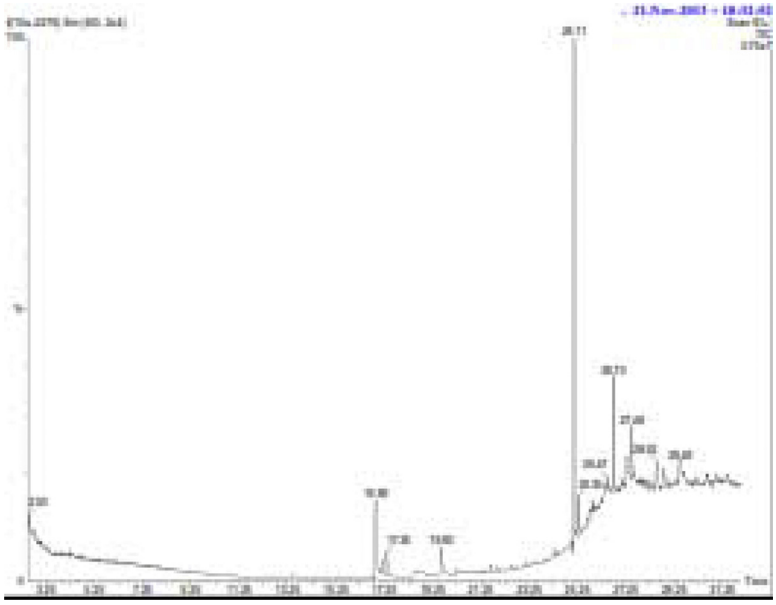

Fig. 6. Gas chromatogram of chemical ingredients of chloroform extract of *A. karoo* root.

3. Results and discussion

Both plant extracts showed broad-spectrum activity, and *Z. mauritiana* extracts had good antimicrobial activity. Ethyl acetate extracts of *Z. mauritiana* leaves caused the maximum zone of inhibition against *S. aureus* (31 ± 2 mm) and the lowest against *E. coli* (13.67 ± 1.53) (Fig. 1). For *A. karoo*, a methanol extract of leaves caused the maximum zone of inhibition against *P. vulgaris* (20.33 ± 1.53 mm) and the lowest against *S. typhi* (10.33 ± 1.53 mm). An ethyl acetate extract of *A. karoo* root caused the maximum zone of inhibition against *S. aureus* (33.3 ± 1.53 mm) and the lowest against *E. coli* (8.67 ± 1.53 mm) (Fig. 2).

A chloroform crude extract of *A. karoo* leaves contained three ingredients: cyclohexanone, 2-methylene-5-(1-methylethyl), 4-methyl-2,4-bis(4′-trimethylsilyloxyphenyl)pentene and trimethyl[4-(1,1,

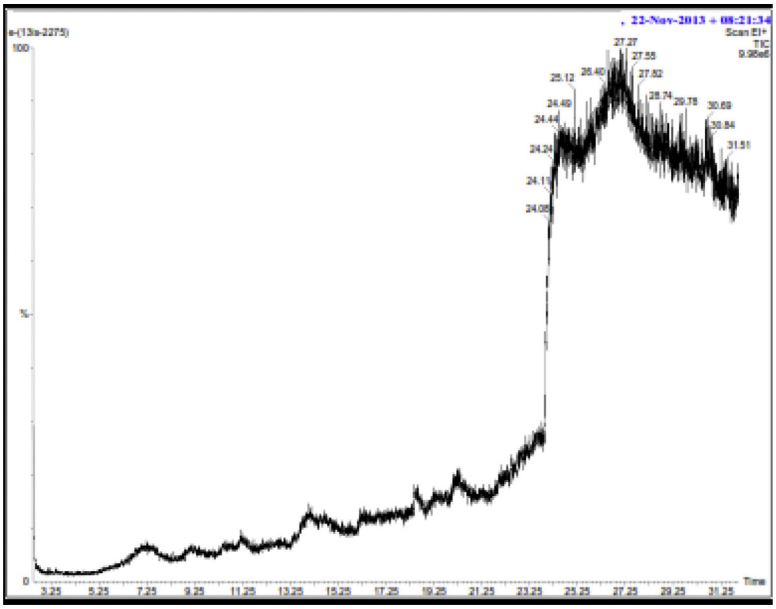

Fig. 7. Gas chromatogram of chemical ingredients of ethanol extract of *A. karoo* root.

3,3,-tetramethylbutyl)phenoxy]silane (Table 1 and Fig. 3). The ethyl acetate extract of *A. karoo* leaves contained six chemicals: methyl-2,4-bis(4′-trimethylsilyloxyphenyl)pentene, cyclotrisiloxane, hexamethyl-*trans*-decalin, 2-methyl-(bicyclo[2.2.1]heptan-2-one, 4,7,7-trimethyl 1s, furan, 2-hexyl-, cyclohexane, 1-methyl-4-(1-methylethenyl)-, *trans*- (Table 1 and Fig. 5). The ethanol extract of *A. karoo* leaves contained eight chemicals: cyclodecene, 1-methyl-acetamide, *N*-(3-imidazol-1-yl-propyl)-2-methoxy-cyclohexene, 1-pentyl-bicyclo[2.2.1]heptan-2-one, 4,7,7-trimethyl-(1r)-trimethyl[4-(1,1,3,3,-tetramethylbutyl)phenoxy] silane, cyclotrisiloxane, hexamethyl,methyl-2,4-bis(4′-trimethylsilyloxyphenyl)pentene, oxamide and *N*-[3-(1-imidazolyl)propyl]-*N*'-methyl (Table 1 and Fig. 4).

The ethyl acetate extract of the roots of *A karoo* contained six chemicals: bicyclo[4.1.0]heptan-3-one, 4,7,7-trimethyl-[1r-(1.alpha.,4.beta.,6.alpha, furan, 2-hexyl-, spiro[5.5]undecane, cyclodecene, 1-methyl-,

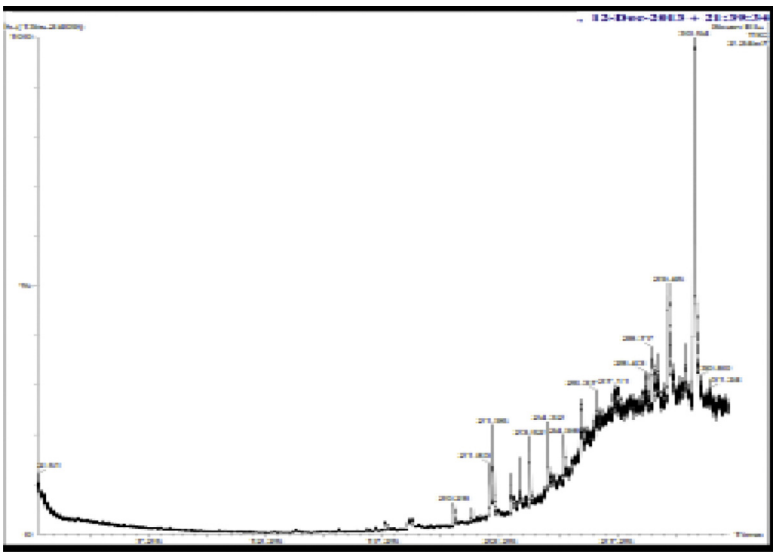

Fig. 8. Gas chromatogram of chemical ingredients of ethyl acetate extract of *A. karoo* root.

cis-decalin, 2-*syn*-methyl-, cyclotrisiloxane, hexamethyl (Table 1 and Fig. 8). The ethanol extract of *A. karoo* roots also contained six chemicals: bicyclo[4.1.0]heptan-3-one, 4,7,7-trimethyl-[1r-(1,alpha.,4.alpha.,6.alpha, cyclodecene, 1-methyl-2-cyclohexen-1-one, 2-methyl-5-(1-methylethyl)-(*S*)-1,2-benzisothiazol-3-amine *tert*-butyldimethylsilyl ether, trimethyl(4-*tert*-butylphenoxy)silane, cyclotrisiloxane, hexamethyl (Table 1 and Figs. 6 and 7).

Most of these compounds have previously been found in other plants. The major compounds are all biologically active molecules and are considered to act in the plants' defence system, as part of a large group of protective molecules called "phytoanticipins" or "phytoprotectants" [12–19]. This is the first report on GC–MS analysis of leaf and root extracts of *A. karoo*.

4. Conclusion

Leaf and root extracts of both *A. karoo* and *Z. mauritiana* had broad-spectrum antimicrobial activity against clinical isolates of bacteria. We conclude that ethyl acetate extracts of *A. karoo* root are a good source of antimicrobial compounds. In future, the bioactive molecule should be isolated and characterized.

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