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Gas Chromatography-Mass Spectrometry profiling, and evaluation of antioxidant and antibacterial activity of *Albizia* spp.

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Abstract. *Ghosh A, Majumder S, Saha S, Sarkar S, Bhattacharya M. 2021. Gas Chromatography-Mass Spectrometry profiling and evaluation of antioxidant and antibacterial activity of* Albizia *spp. Nusantara Bioscience 13: 177-184.* Plant resources have been utilized for human welfare since ancient times. Most of the pharmaceutical industry relies on bioactive molecules from plant resources. This research was carried out to investigate the antioxidant and antibacterial activities of *Albizia* spp. DPPH assay was conducted to investigate the antioxidant activity, and a well-diffusion test by pour plate method was followed for antibacterial assay. Antioxidant activity confirmed that the leaf extracts with chemical contents having solubility in polar solvents showed high free radical scavenging activity. Bark extracts' free radical scavenging activities were lower than the leaf extracts. Leaf extracts of *Albizia procera* showed the highest scavenging activity (94.77% or 100.66 µg AE/ ml), followed by *Albizia odoratissima* (93.52% or 99.23µg AE/ ml), *Albizia lebbeck* (91.68% or 97.13 µg AE/ ml) and *Albizia chinensis* (89.91% or 95.11 µg AE/ ml). GC-MS analysis of leaf and bark extracts revealed that 15 compounds had been reported to have antioxidant activities such as beta-amyrin, phytol, squalene, and vitamin E, and 31 compounds had been reported to have antimicrobial, antibacterial, antifungal, and antiviral activity. The antibacterial activities of leaf and bark extracts of four *Albizia* species showed that leaf extracts of *Albizia* spp. showed better antibacterial activity compared to bark samples which were compatible with the results from GC-MS analysis.

Keywords: *Albizia*, antimicrobial, antioxidant, GC-MS, shade tree

Abbreviations: AO: *Albizia odoratissima*; AC: *Albizia chinensis*; AP: *Albizia procera*; AL: *Albizia lebbeck*; L: Leaf extract; B: Bark extract; GC-MS: Gas chromatography-mass spectrometry; DPPH: 2,2-diphenyl-1-picrylhydrazyl; SA: *Staphylococcus aureus*; BS: *Bacillus subtilis*; EC: *Escherichia coli*; KP: *Klebsiella pneumoniae*

INTRODUCTION

The number of *Albizia* species in tropical and subtropical plains of Asia and Africa is around 150 (Kokila et al. 2013). *Albizia* trees are mainly used as shade trees for tea plantations in North-East India. However, recent research suggests that Albizia spp. from tea gardens can be used for various purposes, including bioactivities other than as shade trees (Ghosh et al. 2020). Different species of the genus *Albizia* contain potential bioactive phytochemicals from various classes of secondary metabolites like saponins, terpenes, alkaloids, and flavonoids (Kokila et al. 2013). Many modern techniques are available and widely accepted for chemical compound analysis. It is well-known that plants produce numerous secondary metabolites with pharmacological or toxicological activities. Several plant species are used as shade trees in tea plantations, such as *Dalbergia sissoo*, *Derris robusta*, *Acacia lenticularis*, *Albizia chinensis*, and *Albizia odoratissima* (Ghosh et al. 2020). Besides their conventionally used as shade trees, bioactive compounds produced by these tree species might have pharmacological activities.

Antioxidant and antimicrobial potentials of some other shade trees in tea plantations, such as *Dalbergia sissoo*, *Derris robusta*, *Acacia lenticularis*, *Leucaena leucocephala*, *Cassia siamea*, *Melia azedarach*, have been reported (Al-Snafi 2017, Paul et al. 2019, Mohammed et al. 2015, Phaiphan et al. 2014, Khatoon et al. 2014), however, antioxidant and antimicrobial activities of *Albizia* spp. (*Albizia chinensis*, *Albizia odoratissima*, *Albizia lebbeck*, and *Albizia procera*) have not been reported yet. Therefore, this study was carried out to determine the bioactivities of four species of *Albizia*. Moreover, the selected plant parts of *Albizia* spp. were collected, and determined their potential bioactivities as antioxidant, antimicrobial, and biochemical properties were by GC-MS analysis.

MATERIALS AND METHODS

Sample collection

Fresh leaves and barks of *Albizia chinensis* (Osbeck)Merr.; *Albizia odoratissima* (L.f.) Benth,; *Albizia lebbeck* (L.)Benth and *Albizia procera*(Roxb.) Benth. were collected from the tea plantation at the University of North Bengal (26º42′47.1′′N 88º20′54.2′′E).

Sample preparation

The leaves and barks of Albizia spp. were cleaned under running water; the surface was dried by dry blotting paper and ground to fine dust in liquid nitrogen (Ghosh et al. 2020). The ground samples (3 grams) were extracted in nine non-polar to polar solvents viz hexane, benzene, chloroform, diethyl ether, ethyl acetate, ethanol, methanol, and water for 48 hours. Then, the extracts were filtered through Whatman no. 1 filter paper and stored in a refrigerator for further analysis. Moreover, being a polar and aprotic solvent, Acetone extract was selected for GC-MS analysis due to its polar and aprotic properties (Julianto and Nurlestari 2018).

Qualitative analysis

Qualitative analysis for the detection of bioactive compounds, i.e., tannin, coumarin, cardiac glycosides, steroid, and flavonoids, was done using the protocols of Ghosh et al. (2020) and Majumder et al. (2021).

Test for flavonoids

A few drops of 10% $FeCl_3$ solution were added to 1 ml leaf and bark extracts. A green or blue color indicated the presence of flavonoids.

Test for coumarin

A few drops of NaOH solution were added to 1 ml leaf and bark extracts. Yellow coloration indicates the presence of coumarin. Next, to test the presence of terpenoid, 250 µl extract was evaporated, the remaining was dissolved in chloroform, and concentrated H_2SO_4 was added from the sidewall of test tubes. The formation of red to reddish-brown coloration at the base confirmed the presence of terpenoids.

Test for cardiac glycosides

0.5 ml of the sample was evaporated and dissolved in 1 ml glacial acetic acid. Next, the side of the test tube added 1 drop of 10% FeCl3 solution, was followed by 1 ml of concentrated H2SO4. The appearance of a brown color ring at the interface indicated the presence of cardiac glycosides.

Test for steroid

0.5 ml of leaf and bark extracts were evaporated and dissolved in 2 ml chloroform, and then 2 ml of concentrated H_2SO_4 was introduced carefully by the sidewall of the test tube. The formation of a red color ring confirmed the presence of Steroids.

Test for tannin

0.5ml leaf and bark extracts were added with a few drops of HNO_3. The reddish to the yellow color of the solution indicated the presence of tannins.

Antioxidant activity (DPPH assay)

Free radical scavenging or antioxidant activity by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was conducted following the protocol of Bhattacharya et al. 2009 and Ghosh et al. 2020. 0.2 ml of leaf and bark extract (500 mg/ml) of *Albizia* spp. was added to 3 ml of 0.2 mM DPPH in methanol. At room temperature, the mixture was vortexed for 30 minutes in the dark. The absorbance was measured at 517 nm by a UV-Vis spectrophotometer. The ascorbic acid (µg/mL methanol) standard curve was taken as a reference. DPPH scavenging activity was expressed as a percentage of inhibition.

***In vitro* antibacterial assay**

Antibacterial assay was done by well diffusion method, following the protocol of (Ghosh et al. 2020). The overnight cultures of two Gram-positive bacteria, i.e., *Staphylococcus aureus* (SA) and *Bacillus subtilis* (BS)] and two Gram-negative bacteria, i.e., *Escherichia coli* (EC) and *Klebsiella pneumoniae* (KP) were used in this study. Leaf and bark extracts of different solvents were dried and dissolved in 1 ml Dimethyl sulfoxide (DMSO) at 500 mg/ml concentration. Mueller Hinton Agar was poured into a Petri dish, solidified, and then inoculated with 200µl inoculum. Nine wells were created with cork borer for 9 different extracts. Then,100µl of each leaf and barks extracts was pipetted into the wells. After that, plates were incubated at 37ºC for 24 hours. Antibacterial assay was determined by measuring the diameter of the inhibitory zone surrounding the well containing the leaf and bark extracts.

Gas Chromatography-Mass Spectrometry analysis

GC-MS analysis was done by following the protocol of (Majumder et al. 2020 and Chakraborty et al. 2021). Acetone extract of bark and leaves of four *Albizia* spp. with a concentration of 25 mg/ml was used for GC-MS analysis. One µL of extracts was injected in the split mode in the instrument (GCMS-QP2010 Plus). The injection temperature was 260 °C, and the interface temperature was set to 270 °C. The ion source temperature was adjusted to 230 °C. Helium was used as carrier gas. The total flow rate was 16.3 ml/min, and the column flow rate was 1.21 ml/min. Mass spectra were recorded at 5 scan/s with a scanning range of 40-650 m/z. Quantification of compounds was done based on their peak areas. The data obtained from GCMS analysis were further analyzed from the available literature.

RESULTS AND DISCUSSION

Qualitative analysis

Qualitative phytochemical analysis shows the presence of valuable groups of phytochemicals in plant extracts. For example, leaf and bark extracts detected organic groups (tannin, coumarin, cardiac glycosides, steroid, and flavonoids) (Table 1).

Antioxidant activity by DPPH assay

Leaf water extracts of four *Albizia* spp. showed the high free radical scavenging activity, out of which leaf extracts of AP showed the highest scavenging activity (94.77% or 100.66 µg AE/ ml), followed by AO (93.52% or 99.23µg AE/ ml), AL (91.68% or 97.13 µg AE/ ml) and AC (89.91% or 95.11 µg AE/ ml). The methanol extract of AP

bark showed the highest DPPH activity (89.16% or 94.25 µg AE/ ml), followed by the water extract of AL bark (88.29 or 93.26 µg AE/ ml) (Table 2).

GC-MS analysis

GC-MS analysis revealed 36 different bioactive compounds (antioxidant and antimicrobial) in 8 different samples (leaf and bark extracts of each plant), as shown in Table 3. GC-MS analysis of leaf and bark extracts revealed 15 compounds with antioxidant and 31 compounds with reported antimicrobial, antibacterial, antifungal, and antiviral activity (Table 3). Among the 15 compounds that showed antioxidant properties, 12 were obtained from leaf extracts and 3 from bark extracts. The 12 compounds are present in the leaf extracts. The 3 compounds, viz. phytol, squalene, and vitamin E, are present in the leaf extract of four species of *Albizia* spp. Seven compounds viz. phytol, squalene, vitamin E, longifolene, 9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z), 9,12-Octadecadienoic acid, methyl ester which have antioxidant activity were obtained from AO leaf extracts. AP contained 6 compounds that have antioxidant activity, viz. Alpha tocospiro B, Beta-amyrin, phytol, squalene, stigmasterol, vitamin E. AC contained 4 antioxidant compounds, i.e., phytol, squalene, vitamin E, bergamot) and AL (4 compounds viz phytol, squalene, tetracontane, vitamin E).

Antibacterial activity

Antibacterial activities of leaf and bark extracts of 4 *Albizia* spp. were screened against 2 Gram-positive and 2 Gram-negative bacteria. The extracts' average inhibitory diameter (cm) varied highly (Table 4.). The bark extracts showed lower inhibitory activity compared to the leaf extracts. Among the leaf extracts, significant inhibition zones were produced; the highest growth inhibition zone was obtained from the ethyl acetate extract of AL-L against EC (1 cm), followed by the water extract of AL-L (0.9 cm). It indicated that the ethyl acetate and the water extract of AL-L had the best antibacterial activity. E.coli was the most susceptible bacteria to those extracts.

Table 1. Phytochemical content of leaf and bark extracts of four *Albizia* spp.

Plants	Solvents	Flavonoids		Cardiac glycosides		Coumarin		Steroid		Tannin	
		Bark	Leaves	Bark	Leaves	Bark	Leaves	Bark	Leaves	Bark	Leaves
Albizia odoratissima	Hexane	L	A	A	VL	A	A	A	A	A	A
(AO)	Benzene	L	A	A	A	A	L	A	L	A	VL
	Chloroform	M	A	L	A	A	A	A	M	L	VL
	Diethyl ether	M	VL	A	A	A	A	A	M	L	VL
	Ethyl acetate	L	M	A	A	A	H	A	M	L	L
	Acetone	M	H	L	A	A	H	A	H	L	M
	Ethanol	H	H	L	VL	A	H	M	H	L	M
	Methanol	M	H	M	VL	A	H	M	M	L	M
	Water	L	A	A	L	A	L	M	L	L	VL
Albizia chinensis	Hexane	L	A	A	A	A	A	A	VL	A	VL
(AC)	Benzene	L	A	A	A	A	L	M	L	A	L
	Chloroform	L	A	A	A	A	L	M	L	L	L
	Diethyl ether	L	VL	A	A	A	A	A	L	L	M
	Ethyl acetate	L	M	A	A	A	H	A	M	VL	M
	Acetone	M	M	L	H	A	H	M	H	VL	H
	Ethanol	M	M	L	H	A	H	A	H	VL	H
	Methanol	M	M	L	M	A	H	M	M	VL	H
	Water	L	VL	A	L	A	H	M	L	VL	H
Albizia procera	Hexane	L	A	A	VL	A	A	A	A	A	VL
(AP)	Benzene	L	A	L	M	A	L	A	L	VL	VL
	Chloroform	L	A	L	L	A	A	M	VL	L	VL
	Diethyl ether	L	A	A	L	A	L	M	VL	L	L
	Ethyl acetate	L	L	L	M	A	H	A	L	L	M
	Acetone	M	L	H	A	A	H	H	VL	M	H
	Ethanol	M	M	M	H	A	H	M	L	M	H
	Methanol	H	M	H	H	A	H	H	L	H	H
	Water	M	A	M	M	A	A	H	VL	M	L
Albizia lebbeck	Hexane	L	A	A	A	A	L	A	L	A	A
(AL)	Benzene	L	A	A	A	A	A	A	L	A	A
	Chloroform	L	A	M	VL	A	A	M	L	L	VL
	Diethyl ether	L	VL	L	VL	A	A	A	L	VL	L
	Ethyl acetate	L	L	L	VL	A	H	M	M	VL	L
	Acetone	M	M	H	VL	A	H	H	M	M	L
	Ethanol	H	M	H	VL	A	H	H	M	M	L
	Methanol	H	A	H	L	A	A	H	VL	H	VL
	Water	H	VL	H	L	A	L	H	VL	H	VL

Note: VL: very low; L: low; M: moderate; H: High; A: absent

Table 2. DPPH scavenging activity (equivalent to Ascorbic acid scavenging activity) of leaf and bark extracts of four *Albizia* spp. dissolved in different solvents

			Hexane	Benzene	Chloroform	Diethyl ether	Ethyl acetate	Acetone	Ethanol	Methanol	Water
AO	Leaf	DPPH assay (%)	13.58	29.42	48.15	77.55	89.19	79.36	75.63	81.34	93.52
		AE (µg/ ml)	7.97	26.06	47.44	81	94.29	83.06	78.81	85.32	99.23
	Bark	DPPH assay (%)	14.36	14.33	22.05	1.78	8.98	25.35	29.99	22.66	12.15
		AE (µg/ ml)	8.86	8.83	17.64	-	2.72	21.41	26.71	18.34	6.34
AC	Leaf	DPPH assay (%)	16.12	15.32	25.04	77.9	78.37	86.52	80.01	81.42	89.91
		AE (µg/ ml)	10.87	9.96	21.06	81.4	81.93	91.24	83.81	85.42	95.11
	Bark	DPPH assay (%)	3.6	42.13	46.15	42.97	39.84	28.11	37.93	21.38	15.28
		AE (µg/ ml)	-	40.56	45.15	41.52	37.95	24.56	35.77	16.88	9.91
AP	Leaf	DPPH assay (%)	21.24	30.24	81.64	85.95	82.38	85.06	88.4	74.46	94.77
		AE (µg/ ml)	16.72	26.99	85.67	90.59	86.51	89.57	93.38	77.47	100.66
	Bark	DPPH assay (%)	34.81	31.6	19.48	11.65	35.54	53.16	88.45	89.16	84.57
		AE (µg/ ml)	6.029	28.54	14.71	5.77	33.04	53.16	93.44	94.25	89.01
AL	Leaf	DPPH assay (%)	9.27	6.79	19.86	58.65	79.45	67.94	61.61	46.14	91.68
		AE (µg/ ml)	3.05	0.22	15.14	59.42	83.17	70.03	62.8	45.14	97.13
	Bark	DPPH assay (%)	44.12	32.28	51.62	31.97	46.16	79.91	79.91	81.32	88.29
		AE (µg/ ml)	42.84	29.32	51.4	28.97	45.17	83.69	83.69	85.3	93.26

Discussion

The results of the DPPH assay confirmed that the leaf extracts with chemical content that tend to be polar showed high free radical scavenging activity. However, the free radical scavenging activity of the bark extracts was lower than the leaf extracts. Leaf extracts of AO contain several compounds which have antioxidant activity, i.e., phytol is present in the highest percentage (5.64%) followed by squalene (5.59%) and 9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z) (5.33%). Leaf extracts of AL contain squalene in the highest percentage (11.39%), followed by vitamin E (9.29%). Leaf extract of AC contains vitamin E in the highest amount (14.74%), followed by squalene (8.7%), while leaf extracts of AP contain vitamin E that present in the highest amount (7.58%), followed by phytol (3.94%). In the bark extracts of AL, 3 compounds have antioxidant activity, namely 2-hydroxy-2-methyl-4-pentanone (diacetone), alpha-bisabolol oxide, and gamma-sitosterol. Diacetone (2-hydroxy-2-methyl-4-pentanone is a compound that has antioxidant properties, and this compound is present in the bark extracts of AO (42.39%); AL (35.52%); AC (57.84%) except the bark extract of AP. The high percentage of compounds with antioxidant properties could affect the free radical scavenging activity of the extract.

Based on the results of the GC-MS analysis, 31 compounds were successfully identified from the leaf and bark extract. There were 25 compounds, and 12 compounds were identified from the leaf and bark extracts, respectively. Based on the literature study, there are 17 compounds, such as oleoyl chloride; dehydroabietic acid; eucalyptol; phenol; phytol; squalene, etc., have antimicrobial activity, 17 compounds, such as beta-Amyrin; longifolene; squalene; phytol; methyl commate B; gamma-linolenic acid, methyl ester, etc. have antibacterial activity, 11 compounds such as eucalyptol; 13-Hexyloxacyclotridec-10-en-2-one; 1,2benzenedicarboxylc acid, diethyl ester; acetophenone; beta-amyrone, etc. have antifungal activity and 4 compounds viz. Nonacosane, methyl commate B, lupenone, and stigmasterol have antiviral activity (Table 3). Nine AO, AL, and AP compounds and 8 compounds of AC showed antimicrobial activity. Eleven compounds had antibacterial activity in the leaf and bark extracts of AO; 7 compounds had antibacterial activity in the leaf and bark extracts of AL, followed by AP with 6 compounds and AC with 5 compounds. There are 6 compounds reported as antifungal in both AC and AO and 4 in AL and AP. There are 3 compounds reported as antiviral compounds in the leaf and bark extracts of AP, followed by AO and AL with 1 compound. Antimicrobial compound, namely 1-Heptanol, 2-propyl was detected in the highest percentage (43.22% in the bark extract of AP), followed by squalene (11.39% in the leaf extract of AL and 8.7% in the leaf extract AC) and phytol (5.64% in the leaf extract of AO). Antibacterial compound, squalene is present with the highest percentage (11.39% in the leaf extract of AL), followed by gamma-sitosterol (9.98% in the bark extract of AL); squalene (8.7% in the leaf extract of AC); phytol (5.64% in leaf extract of AO); methyl commate B (5.59%in leaf extract of AP). Antifungal compound, 13-Hexyloxacyclotridec-10-en-2-one is present with the highest percentage (23.37% in the bark extract of AL and 5.99% in the bark extract of AO), followed by phytol (5.64% in the leaf extract of AO and 3.94% found in the leaf extract of AP). Antiviral compounds, Lupenone is present with the highest percentage (8.72% in the leaf extract of AL); followed by methyl commate B (5.59%); Lupenone (1.38%), and stigmasterol (1.06%) in the leaf extract of AP.

Table 3. Bioactive compounds from *Albizia* spp. detected by GC-MS analysis

Compounds	Peak area %								Antioxidant and antimicrobial activity	References
	AO		AL		AC		AP			
	L	B	L	B	L	B	L	B		
alpha-Tocospiro B							0.99		Antioxidant	Deora and Bano 2019
beta-Amyrin							0.45		Antioxidant	Elangovan et al. 2015
									Antibacterial	
									Antifungal	Elfadil et al. 2015
Phytol	5.64		2.83		1.77		3.94		Antioxidant	Costa et al. 2016
									Antimicrobial	Pejin et al. 2014
									Antifungal and antibacterial	Hema et al. 2017
Squalene	5.59		11.39		8.7		1.65		Antioxidant	Rautela et al. 2017
									Antimicrobial	Elakkiya and Murugaiah 2015
									Antibacterial	
Stigmasterol							1.06		Antioxidant	Manorenjitha et al. 2013
									Antiviral	
									Antimicrobial	Yinusa et al. 2014
Tetratetracontane			5.73						Antioxidant	Jayalakshmi et al. 2018
Vitamin E	3.54		9.29		14.74		7.58		Antioxidant	Filippo and Cannas 2006
+ Longifolene	1.04								Antioxidant	Hadi et al. 2016
									Antibacterial	Himejima et al. 1992
									Antimicrobial	
9,12,15-Octadecatrienoic acid, methyl ester, Z, Z, Z	5.36								Antioxidant	Godwin et al. 2015
									Antibacterial	
Bergamiol					1.29				Antioxidant	Duke 1992-2016
9,12-Octadecadienoic acid, methyl ester	2.13								Antioxidant	Oloyede 2012
									Antifungal and antibacterial	Godwin et al. 2015
2-hydroxy-2-methyl-4-pentanone diacetone alcohol		42.39		35.52		57.84			Antioxidant	Srinivasan et al. 2014
alpha-Bisabolol oxide				0.88					Antioxidant	Thenmozh and Rajan 2015
									Antimicrobial	
									Antibacterial	Móricz et al. 2012
gamma-Sitosterol				9.98					Antioxidant	Akpuaka et al. 2013
									Antibacterial	
Longiborneol	3.14	3.45							Antioxidant	Ahmadvand et al. 2014
									Antibacterial	Ramalakshmi and Muthuchelian 2011
									Antifungal	
1-Heptanol, 2-propyl-	3.69	4.54	5.81	3.77	8.7	5.67	1.54	43.22	Antimicrobial	Lakshmi and Rajalakshmi 2011
9-Octadecenamide, Z-							0.25		Antibacterial	Idan et al. 2015
13-Hexyloxacyclotridec-10-en-2-one		5.99		23.37	1.34		3.38		Antifungal	Hameed et al. 2018
beta-amyrone							0.63		Antifungal	Elfadil et al. 2015
Gamma-Linolenic acid, methyl ester	1.4								Antibacterial	Pubchem 2021
Cyclopentadecanone, 2-hydroxy-	0.61								Antimicrobial	Oyedoh et al. 2020
Dehydro-abietic acid			1.52		1.42				Antibacterial	Fallarero 2013
Dehydro-abietyl amine	0.81								Antibacterial	Rao et al. 2008
									Antifungal	
Acetophenone					1.27				Antifungal	Groenhagen et al. 2013
Lupenone			8.72				1.38		Antiviral	Xu et al. 2018
Methyl commate B							5.59		Antibacterial	Arora and Kumar, 2017
									Antimicrobial	
									Antiviral	
									Antiparasitic	
Neophytadiene	4.94		1.81		1.27		2.26		Antibacterial	Lahigi et al. 2011
									Antimicrobial	Mendiola et al. 2008
Pentafluoropropionic acid, heptadecyl ester							0.4		Antimicrobial	Pandey et al. 2011
Phenol		4.63		3.98			1.17		Antimicrobial	Pubchem 2021
Bis(2-Ethylhexyl) phthalate	0.62				0.68				Antimicrobial	Srinivasan et al. 2009
1,2-benzene dicarboxylic acid, diethyl ester	2.55	2.55		2.74	1.45	2.62			Antibacterial	Srinivasan et al. 2009
									Antimicrobial	Premjanu and Jaynthy 2014
									Antifungal	Moustafa et al. 2013
1,4-dimethyl-3-2-methyl-1-propenyl-4-vinyl							3.33		Antimicrobial	Jalpa et al. 2015
Cyclopentasiloxane, decamethyl-						0.81			Antimicrobial	Mahmud et al. 2018
									Antifungal	
Eucalyptol				0.49		0.72			Antimicrobial	Naveed et al. 2013
									Antifungal	Jiang et al. 2020
Oleoyl chloride				0.80					Antimicrobial	Amaechi 2018
Nonacosane		0.86							Antibacterial	Akpuaka et al. 2013
									Antiviral	Mathur et al. 2014

Table 4. The average inhibitory diameter (cm) of leaf and bark extracts of *Albizia* spp

Solvent extract	Bacteria	AO-L	AL-L	AC-L	AP-L	AO-B	AL-B	AC-B	AP-B
Hexane	EC	0.5	0.3	0.4	0.5		0.15		0.175
	KP				0.2				
	BS								
	SA	0.2	0.075						
Benzene	EC	0.2		0.3		0.2	0.1		
	KP		0.5						
	BS	0.22							
	SA	0.05	0.1		0.3				
Chloroform	EC	0.5		0.4		0.4	0.25		
	KP				0.45				
	BS	0.7					0.15		
	SA	0.2			0.2				
Diethyl ether	EC		0.4						
	KP								
	BS	0.5							
	SA			0.2			0.1		
Ethyl acetate	EC		1	0.6	0.35				
	KP								
	BS	0.2							
	SA	0.1	0.35		0.15				
Acetone	EC	0.7			0.3		0.3		
	KP						0.35		
	BS						0.2		
	SA				0.4		0.35		
Ethanol	EC	0.3		0.7	0.3		0.15		
	KP				0.2		0.2		
	BS								
	SA		0.35	0.2			0.3		
Methanol	EC	0.4		0.7	0.3		0.15	0.15	
	KP						0.05		
	BS						0.1		
	SA				0.4		0.25		
Water	EC	0.35	0.9	0.5	0.5				
	KP								
	BS		0.3		0.2				
	SA								

The results of the antibacterial assay showed that the extracts were more potent against Gram-negative bacteria such as *E. coli* which was inhibited by almost all extracts with the various polarity of solvents. However, unlike Gram-positive bacteria. Regarding leaf samples, ethyl acetate and water extracts showed remarkably good results, mainly for AL, followed by AC and AO. Regarding bark samples, acetone extracts showed a maximum inhibition zone. The results show a similar pattern of more inhibition potential against Gram-negative bacteria than Gram-positive ones.

The remarkable outcome of this research is the detection of potential antioxidant and antimicrobial components that are soluble in polar solvents. The selection of acetone extract used for GC-MS analysis is due to the capability of acetone to extract polar and non-polar components. The present study on *Albizia* spp., which is usually used as shade trees in tea plantations, showed the possibility of utilizing their metabolites as a source of antioxidant or antibacterial agents. It can increase the added value of *Albizia* spp, which may have a beneficial step toward the economic stability of the tea industry.

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