Advances in Microbiology, 2016, 6, 691-698
Published Online August 2016 in SciRes. http://www.scirp.org/journal/aim
http://dx.doi.org/10.4236/aim.2016.69068

Scientific Research Publishing

Evolution of Antimicrobial, Antioxidant Potentials and Phytochemical Studies of Three Solvent Extracts of Five Species from *Acacia* Used in Sudanese Ethnomedicine

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Received 28 March 2016; accepted 8 August 2016; published 11 August 2016

Abstract

The ethanol, chloroform and acetone extracts of five species from *Acacia* (*Acacia albidia* stems, *Acacia mellifera* aerial parts, *Acacia nubica* aerial parts, *Acacia seyal var. seyal* stems and *Acacia tortilis* aerial parts) were investigated for their antimicrobial activity against two standard bacterial strains of Gram +ve bacteria (*Staphylococcus aureus* (*ATCC* 25923)), Gram −ve bacteria (*Pseudomonas aeruginosa* (*ATCC* 27853)) and standard fungi *Candida albicans* (*ATCC* 90028) using the agar-plate well diffusion method. The chloroform extract was inactive compared to ethanol and acetone extracts. But ethanol extracts showed the maximum antimicrobial activity against the test organism. Amongst the plant species screened, ethanol extract of *Acacia seyal* stems showed maximum inhibitory activity (38 mm) and (37 mm) against *Staphylococcus aureus* and *Candida albicans*, respectively. The ethanol, chloroform and acetone extracts of *Acacia mellifera* (aerial parts) did not show any activity against the test organisms. Cholorophorm and acetone extracts via DPPH, the radical scavenging activities were found to be 91 ± 0.03, 88 ± 0.01 and 85 ± 0.04, respectively. The results of phytochemical screening showed that all extracts of studied plant contain flavonoids, saponins, terpenoids, steroids, alkaloids, phenols and tannins.

How to cite this paper: Abdllha, H.B., Mohamed, A.I., Almoniem, K.A., Adam, N.I., Alhaadi, W., Elshikh, A.A., Ali, A.J., Makuar, I.G., Elnazeer, A.M., Elrofaei, N.A., Abdoelftah, S.F. and Hemidan, M.N. (2016) Evolution of Antimicrobial, Antioxidant Potentials and Phytochemical Studies of Three Solvent Extracts of Five Species from *Acacia* Used in Sudanese Ethnomedicine. *Advances in Microbiology*, **6**, 691-698. http://dx.doi.org/10.4236/aim.2016.69068

Keywords

Antimicrobial, Antioxidant, Phytochemical, Sudanese Ethnomedicine *Acacia* Species

1. Introduction

According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies [1].

Some antibiotics have become almost obsolete because of drug resistance and consequently new drugs must be sought for. Herbal treatment is one possible way to treat diseases caused by multidrug resistant bacteria. The use of plant extracts and phytochemicals, with known antibacterial properties, may be of immense importance in therapeutic treatments. In the past few years, a number of studies have been conducted in different countries to prove such efficiency [2].

Acacia is the most significant genus of family: Leguminosae, first of all described by Linnaeus in 1773. It is estimated that there are roughly 1380 species of *Acacia* worldwide, about two-thirds of them native to Australia and rest of spread around tropical and subtropical regions of the world [3] [4]. The aim of this study is to investigate the antimicrobial, antioxidant potentials and phytochemical studies of five species from *Acacia* to ascertain the rationale for its use in traditional medicine.

2. Material and Method

2.1. Plant Material

The plants used in this study were collected from Khartoum university-faculty of agriculture, *Acacia seyal* was collected from local market. This plant was identified in the Botany department, Faculty of science and technology, Omdurman Islamic University by Prof. Hatil Hashim Al-Kamali and by comparison with herbarium of the department. The plant was spread and dried in the shade for three weeks and then pulverized with mechanical grinder.

2.2. Preparation of Plant Extracts

Each of the coarsely powdered plant material (200 g) was exhaustively extracted for 24 hours with 1 liter of ethanol in conical flasks. The ethanolic extract was filtered and evaporated. The extracted plant material was then air-dried, repacked in conical flask and exhaustively extracted with 800 ml of chloroform extract was filtered and evaporated. The extracted plant material was then air-dried and repacked in conical flask and exhaustively extracted with 600 ml of acetone. Each residue was weighed and determined by sensitive balance.

2.3. Preparation of Bacterial Suspensions

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37˚C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8 - 10^9 C.F.U./ml. The suspension was stored in a refrigerator at 4˚C till used.

The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique [5]. Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes on drop of the appropriate dilutions were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37˚C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units

per ml suspension (C.F.U./ml).

Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

2.4. Preparation of Fungal Suspension

The fungal cultures was maintained on Sabouraud dextrose agar, incubated at 25˚C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100 ml of sterile normal saline, and the suspension was stored in the refrigerator until used.

2.5. *In Vitro* Testing of Extracts for Antimicrobial Activity

2.5.1. Testing for Antibacterial Activity

The cup-plate agar diffusion method was adopted according to [6] with some minor modifications to assess the antibacterial activity of the prepared extracts.

One ml of the standardized bacterial stock suspension 10^8 - 10^9 C.F.U/ml were thoroughly mixed with 100 ml of sterile molten nutrient agar which was maintained at 45˚C. 20 ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes.

The agar were left to dry and in each of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed.

Alternate cups were filled with 0.1 ml sample of each extracts using automatic Microlitre-pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37˚C for 18 hours.

Two replicates were carried out for each extracts against each of the test organisms. Simultaneously addition of the respective solvents instead of extracts was carried out as controls. After incubation, the diameters of the resultants and growth inhibition zones were measured, averaged and the mean values were tabulated.

2.5.2. Testing for Antifungal Activity

The same method as for bacteria was adopted. Instead of nutrient agar, Sabouraud dextrose agar was used. The inoculated medium was incubated at 25˚C for two days for the *Candida albicans.*

2.6. Antioxidant Activity

The antioxidant activity has been carried out using two assays:

2.6.1. DPPH Free Radical Scavenging Activity

The DPPH radical scavenging was determined according to the method of [7], with some modification. The test samples were allowed to react with 2.2 di(4-tretoctylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37˚C in 96-wells plate. The concentration of DPPH was kept at (300 μM). The test sample was dissolved in DMSO while DPPH was prepared in ethanol. After incubation decrease in absorbance was measured at 517 nm using multiplate reader spectrophotometer. Percentage of radical scavenging activity of the sample was determined in comparison with a DMSO treated control. All tests were conducted triplicate.

2.6.2. Iron Chelating Activity Assay

The iron chelating ability was determined according to the modified method of [8], in which the Fe^{2+} was monitored by measuring the formation of ferrous ion-ferrozine complex. The experiment was carried out in 96 micrometer plate. The plant extract was mixed with $FeSO_4$. And the reaction was initiated by adding 5 mM ferrozine. The mixture was shaken, left at 25˚C for 10 min, and finally the absorbance was measured at 562 nm, using multi-plate spectrophotometer. EDTA was used as positive control, and DMSO as control. All tests were done in triplicate.

2.7. Phytochemical Screening of Extracts

The ethanol, chloroform and acetone extracts were used for the detection of the following tests according to standard methods. Described [9] [10].

2.7.1. Phenols
Two ml of extract was added to one ml of distilled water and warmed at 45˚C - 50˚C. Then 2 ml of 3% $FeCl_3$ was added. Appearance of green or blue color indicate the presence of phenols.

2.7.2. Flavonoids
One ml of extract was added to one ml of 10% KOH. It was gently shaken. Appearance of yellow color indicated the presence of flavonoids.

2.7.3. Tannins
One ml of extract was added to one ml of 3% $FeCl_3$. A greenish black precipitate indicated the presence of tannins.

2.7.4. Alkaloids
One ml of Dragendorff reagent was added to 1 ml of filtrate. The formation of cloudy orange was observed.

2.7.5. Terpenoids and Steroids
Five ml of extract was mixed in two ml of chloroform. Then 3 ml concentrated sulphuric acid was carefully added to observe a reddish brown coloration between upper and lower layer was observed.

2.7.6. Saponins
Approximately 0.2 ml of extract was mixed with 5 ml of distilled water. Mixture was shaken vigorously for 5 min. Persistence of foams indicated the presence of saponins.

3. Results and Discussion

The mean of diameters of the growth inhibition zone produced by extracts of five species of *Acacia* on the standard strains is shown in **Table 1**. The results of antimicrobial tests were interpreted as active (>18 mm), moderately active (14 - 18 mm) and inactive (<14 mm) [11] [12]. The ethanol extract of *A. seyal* stems showed the highest inhibitory activity (38 mm) and (37 mm) against *Staphylococcus aureus* and *Candida albicans* respectively and had the highest antioxidant activity with 91 ± 0.03 using DPPH assay, but other extracts had less activity (**Table 2**), followed by acetone extract which showed the good activity (30 mm) against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. These might be due to the presence of alkaloids, flavonoids, steroids and trepenoids in ethanol extracts, and alkaloids, flavonoids, saponins, tannins, steroids, trepenoids and phenols in acetone extracts (**Table 3**).

Table 1. Antimicrobial activities of *Acacia* species against standard organisms.

Plants	Extracts	Concentration (%)	Test organism		
			MIDZ (mm)		
			P.a.	***S.a.***	***C.a.***
***A. seyal* (stems)**	**Ethanol**	10%	35	38	37
		5%	30	30	35
		2.5%	29	28	30
		1.25	25	20	24
		Control	-	-	-
	Chloroform	10%	-	-	-
		5%	-	-	-
		2.5%	-	-	-
		1.25	-	-	-
		Control	-	-	-
	Acetone	10%	30	30	29
		5%	28	29	28
		2.5%	22	23	25
		1.25%	19	20	20
		Control	-	-	-

Continued

A. albida **(aerial parts)**	**Ethanol**	10%	20	-	20
		5%	18	-	19
		2.5%	13	-	17
		1.25%	-	-	15
		Control	-	-	-
	Chloroform	10%	-	-	-
		5%	-	-	-
		2.5%	-	-	-
		1.25%	-	-	-
		Control	-	-	-
	Acetone	10%	20	-	-
		5%	18	-	-
		2.5%	15	-	-
		1.25%	-	-	-
		Control	-	-	-
A. tortilis **(aerial parts)**	**Ethanol**	10%	20	20	23
		5%	17	19	20
		2.5%	-	18	17
		1.25%	-	-	-
		Control	-	-	-
	Chloroform	10%	-	-	-
		5%	-	-	-
		2.5%	-	-	-
		1.25%	-	-	-
		Control	-	-	-
	Acetone	10%	23	18	25
		5%	20	-	23
		2.5%	15	-	19
		1.25%	-	-	9
		Control	-	-	-
A. nubica **(aerial parts)**	**Ethanol**	10%	31	25	27
		5%	28	22	23
		2.5%	22	19	20
		1.25%	19	-	16
		Control	-	-	-
	Chloroform	10%	25	-	-
		5%	23	-	-
		2.5%	20	-	-
		1.25%	-	-	-
		Control	-	-	-
	Acetone	10%	29	23	25
		5%	25	20	20
		2.5%	22	19	19
		1.25%	15	-	18
		Control	-	-	-
A. mellifera **(aerial parts)**	**Ethanol**	10%	-	-	-
		5%	-	-	-
		2.5%	-	-	-
		1.25%	-	-	-
		Control	-	-	-
	Chloroform	10%	-	-	-
		5%	-	-	-
		2.5%	-	-	-
		1.25%	-	-	-
		Control	-	-	-
	Acetone	10%	-	-	-
		5%	-	-	-
		2.5%	-	-	-
		1.25%	-	-	-
		Control	-	-	-

MDIZ = Mean Diameter of Inhibition Zone = average of two replicates in millimeters, Tested conc of extract (0.1 ml/well). *S.a*. = *Staphylococcus aurous, P.s. = Pseudomonas aeruginosa, C.a. = Candida albicans.*

Table 2. Antioxidant activity of ethanol, chloroform and acetone extracts of *Acacia* species.

Plants species	Solvents	Results
***A. seyal* (stems)**	Ethanol	91 ± 0.03
	Chloroform	88 ± 0.01
	Acetone	85 ± 0.04
***A. nubica* (aerial parts)**	Ethanol	83 ± 0.02
	Chloroform	28 ± 0.20
	Acetone	79 ± 0.03
***A. tortilis* (aerial parts)**	Ethanol	83 ± 0.02
	Chloroform	42 ± 0.7
	Acetone	82 ± 0.04
***A. albida* (aerial parts)**	Ethanol	89 ± 0.05
	Chloroform	12 ± 0.04
	Acetone	81 ± 0.04
***A. mellifera* (aerial parts)**	Ethanol	37 ± 0.08
	Chloroform	9 ± 0.10
	Acetone	49 ± 0.01

Table 3. Phytochemical s*tudies* of ethanol, chloroform and acetone extracts of *Acacia* species.

Plants species	Solvents	Class of chemical compounds						
		Alkaloids	Flavonoids	Saponins	Tannins	Steroids	Terpenoids	Phenols
A. albida	**Ethanol**	−	+	−	+	+	+	+
	Chloroform	+	−	+	−	+	+	+
	Acetone	+	+	−	−	+	+	−
A. mellifera	**Ethanol**	+	−	−	−	+	+	−
	Chloroform	+	−	−	−	+	+	−
	Acetone	+	+	+	−	+	+	−
A. nubica	**Ethanol**	+	+	+	+	+	+	+
	Chloroform	+	−	+	−	+	+	−
	Acetone	+	+	+	+	+	+	+
A. seyal	**Ethanol**	+	+	−	−	+	+	−
	Acetone	+	+	+	+	+	+	+
A. tortilis	**Ethanol**	+	+	−	−	+	+	−
	Chloroform	+	+	+	−	+	+	−

+ve = Present, −ve = Absent.

[13]-[16] found that some compounds isolated from different plants such as flavonoids, tannin, quinines and coumarins had been extracted and found to be of inhibitory effect on numerous bacteria strains as well as fungi and yeast. Alkaloids had been demonstrates to have high antibacterial activity against Gram-negative and Gram positive bacteria [17]. Among the extracts tested acetone extract showed antimicrobial activity against at least one strains, while chloroform extract was not active against any of the test organisms. This means that the plant part used and the type of extraction might have resulted in nil activity in the test performed in this study. Some of these plants were also screened previously against other test strains [18] [19] and were found to exhibit similar results to those obtained in this study with varying degree in potency. The differences in potency may be due to locality of the plant species, time of collection of the plant sample, storage condition different sensitivity of the test strains and method of extraction [20].

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