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Phytochemical and Antibacterial Screening of Aqueous and Hexane Extracts of *Acacia nilotica* Fruit against Bacterial Species from Water Isolates

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Abstract

An aqueous and hexane (hot and cold) extracts of Acacia nilotica fruit were evaluated for their phytochemical properties and antibacterial activities. The phytochemical screening revealed the presence of tannins, co-anthraquinores, alkaloids, flovonoids, saponin, terpenoids and glycosides. The antibacterial activities of extracts were performed on water isolates collected randomly from different sites of Khartoum State. The bacteriological analysis of water isolates showed the presence of Salmonella, Bacillus, Pseudomonas, Staphylococcus, Micrococcus and Corynebacterium. Both aqueous and hexane extracts exhibited antibacterial activities against the test bacteria, and the aqueous extract was more effective than the hexane (hot and cold). The salmonella was the most sensitive bacteria to the all extracts.

Keywords: *Acacia nilotica*, Antibacterial activity, Phytochemical properties, Tannins, Khartoum.

1. INTRODUCTION

Plants have great significance due to their nutritive value and are also a major source of medicines. Plants ser ed mankind throughout the history of human civilization (Baliek and Palil, 1996). From

ancient times different parts of medicinal plants have been used to cure specific ailments. Medicinal plants are also used extensively by tribal people worldwide (Prashant *et al*., 2011). The World Health Organization (WHO) supports the use of medicinal plants, provided it is proven to be efficacious and safe (WHO, 1995).

Acacia is a genus of shrubs and trees belonging to the subfamily Mimosoileae. Studies indicate that the pod extract showed antibiotic activity against, *Bacillus subtilis, Staphylococcus albus, Streptococcus faecalis* and *Escherichia coli* (Shanab, 2007).

Water is essential for life on earth and safe water is a pre-condition for health and development and basic human right. In many countries around the world, including Sudan, some drinking water supplies have been contaminated which has impacted on the health and economic status of the population. This contamination may result in an increase risk of disease transmission to humans (Glidreich and L-Chevallier, 1999).

The objective of this study was to evaluate the phytochemical constituents of an aqueous and hexane pod extracts of *Acacia nilotica* against some microorganism collected from water isolates.

2. MATERIALS AND METHODS

2.1 Collection of plant materials

Fresh pods of *Acacia nilotica* subsp. tomentosa were collected in 2017 from Sennar State in Sudan. The pods were identified by the Biotechnology Laboratory of Khartoum University. The pods were washed under running tap water and then air dried in the laboratory and ground into fine powder with an electric blender.

2.2 Collection of water samples

Fifteen samples of drinking water were randomly collected from several localities of Khartoum State. The samples were collected in sterile glass bottles of 200ml capacity. The caps of the bottle covered with aluminum foil and sterilized at 160^{o}C. The samples then were taken for bacteriological testing according to Monica (2008).

2.3 Determination of the total viable count bacteria

It was carried out by the use of Pour plate count method as described by Harrigan (1998). 1ml of each dilution was transferred into sterile Petri dishes. 15ml of sterile melted plate agar were added to each plate. The inoculums were mixed with medium and allowed to solidify and the plates were incubated at 37^{o}C for 48 hours. A colony counter was used to count the viable bacterial colonies. The count was expressed as colony forming units (CFU) ml. Preparation of aqueous extract, hot and cold hexane extract were carried out according to Bhakat and Sen (2008).

2.4 Identification of water isolates

For determination the genus of bacterium isolates, some important biochemical test, such as catalase test, oxidase test, gram stain test, motility test, endospore staining test, oxidation fermentation test and glucose acid test were done.

From 15 samples of drinking water, 33 isolation were carried out, 6 genus were determined from the identification of bacterial isolates. 17 isolates were bacillus, 2 were enterobacteria, 1 was pseudomonas, 9 were staphylococcus, 3 were corynebacterium and 1 was micrococcus.

Preparation of aqueous extract, hot and cold hexane extract were carried out according to Bhakat and Sen (2008).

2.5 Preparation of aqueous extract

100g of the powdered sample was transferred into 200ml distilled water and allowed to soak for 72 hours with shaking at intervals of time to ensure that the active substances were extracted. Then the extract was filtered on Whatman's No. 1 filter paper, transferred into sterile bottle and stored in a refrigerator at 4^{o}C until used.

2.6 Preparation of hot hexane extract

100g of the powdered sample was weighted and replaced in custobana, and complete soxhlet apparatus, and 150ml of hexane 95% was added for 8 hours, then the sample was taken out and the separation was done in sterile bottle and stored in a refrigerator at 4^{o}C until used.

2.7 Preparation of cold hexane extract

100g of the powdered sample was weighted and replaced in sterile bottle. 10ml of hexane 95% was added and shacked well from time to time. After 72 hours the extract was filtered on Whatman's No. 1 filter paper, then transferred into sterile bottle and stroed in a refrigerator at 4^{o}C until used.

2.8 Preparation of concentration extracts

Different concentrations of the extracts were prepared by dilution (100mg/ml, 75mg/ml, 50mg/ml and 25mg/ml).

2.9 Antimicrobial screening

The antibacterial activities of plant extracts were determined according to Hugo and Russel (1984). Using agar well diffusion method. Six water isolates were tested. Four of them were gram-positive and two were gram-negative. The bacterial isolates collected in slant of nutrients agar, sub-cultured into prepared nutrients broth and incubated at 37^{o}C for 24h and standardized to 0.5 mcfar land seals (10^{8} cfu/ml) in a prepared normal saline. Into prepared nutrient agar each plate was inoculated with bacterial suspension using a sterile loop. Four wells were made in the plates using a sterile tip (7mm) in diameter. Each of the aqueous and hexane concentration extracts were transferred into the wells with micropipette then allowed to stand for 30 minutes at room temperature for proper diffusion. The plates incubated at 37^{o}C for 24h. A control was set up in parallel. After 24h clear zones of inhibition were measured and compared with that of the standard control. For strains the assay was triple replicated and the mean was taken.

2.10 Phytochemical screening

The phytochemical screening tests were carried out on the aqueous extract and hexane extract (hot and cold) using standard methods to identify the constituents as described by Harbone (1973).

2.10.1 Test of tannins

1g of the powdered sample was boiled with 20ml distilled water for five minutes in a water bath and was filtered while hot. 1ml of cool filtered sample was distilled to 5ml. distilled water and a few drops (2-

3) of 10% ferric chloride were added to observe any formation of precipitates and any colour change. Abolish-black or brownish green precipitate indicated the presence of tannins.

2.10.2 Test for flavonoids

1g of the powdered sample was boiled with 10ml of distilled water for five minutes and filtered while hot, few drops of 20% sodium hydroxide solution were added to 1ml of cooled filtrate. A change to yellow colour, which on addition of acid changed to colourless, indicated the presence of flavonoids.

2.10.3 Test for terpenoids

5ml of extract was mixed in 2ml of chloroform; 3ml of concentrated H_2SO_4 was added to form a layer. A reddish brown precipitate appearance at the inner face indicated the presence of terpenoids.

2.10.4 Test for saponins

3ml of the aqueous solution of the extract were mixed with 10ml of distilled water in a test tube, then Stoppard and shaken vigorously for 5 minutes. It was allowed to stand for 30 minutes and observed for honey comb forth, which was indicating of the presence of saponins.

2.10.5 Test for alkaloids

1g of powdered sample was boiled with 10ml dilute hydrochchloric acid on a water bath and filtered. The pH was adjusted with ammonia to about (6-7). A small quantity of the Mayers reagents was added to 0.5ml of filtrate in a test tube and observed the formation of a yellow cream precipitate which indicates the presence of alkaloids.

2.10.6 Test of glycosides

5ml of the extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution, this was under played with 1ml of concentrated sulphruic acid. A brown ring at the interface indicated the deoxy sugar characteristics of cardenolides. A violet ring may appear below the ring, while in the acetic acid layer a greenish ring may be formed.

2.10.7 Test for co-anthraquinones

1g of the powdered sample was boiled with 2ml of 10% hydrochloric acid for 5 minutes, then filtered while hot. The filtrate was allowed to cool, then was partitioned against equal volume of chloroform and the chloroform layer was transferred into clean dry test tube using a clean pipette. Equal volume of 10% ammonia solution was added into the chloroform layer, which was shaken and allowed to separate. The separated aqueous layer was observed for any colour change, delicate rose, pink colour showed the presence of co-anthraquinones.

2.10.8 Test for carotenoids

1g of the sample was extracted with 10ml of chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85% sulphuric acid was added. A blue colour at the interface showed the presence of carotenoids.

2.10.9 Test for proteins

0.5mg of the extract and equal volume of 40% NaOH solution and two drops of one percent copper sulphate solution was added. The appearance of violet colour indicates the presence of protein.

2.10.10 Test for carbohydrate

1ml of aqueous solution of the extract and 1ml of Borfoed's regent were added into a test tube, heated in a water bath for 2 minutes. A red precipitate showed the presence of monosaccharide.

2.10.11 Test for reducing sugars

1ml of the aqueous solution of the extract was hydrolyzed by boiling with 5ml of dilute hydrochloric acid. This was neutralized with sodium hydroxide solution. The Fehlings test was repeated as indicated above and the tube was observed for brick-red precipitate that indicated the presence of reducing sugar.

3. RESULTS AND DISCUSSION

The results from Table (1) indicated the presence of Bacillus, Staphylo-coccus, Enterobacerium (Salmonella), Corynebacterium, Micrococcus and Pseudomonas from water isolates which were taken

randomly from different sites in Khartoum State. These results indicated that the water was exposed to contamination, which constitute a threat to human health.

The phytochemical analysis of *Acacia nilotica* pod (Table 2) showed the presence of various constituents including tannin, free anthraquinones, alkaloids, saponins, flavonoids, carbohydrates, terpenoids, glycosides and carotenoids. Similar results were obtained by Auwal *et al.* (2014) who found the presence of carbohydrates, saponins, glycosides and flavonoids in *Acacia nilotica* pod.

The phytochemical analysis (Table 2) showed that the concentration of tannins were high (++++) in the aqueous extract, but in hexane extract were moderate concentration (+++). The results also showed the absence of free anthraquinones in both aqueous and hexane extract.

Table (1): The biochemical identification of bacteria in water isolates

Genus / Biochemical test	Ba	St	En	Co	Mi	Ps
Gram staining	+	+	-	+	+	-
Shape	Rod	Sphere	Rod	Rod	Sphere	Rod
Endospore staining	+	-	-	-	-	-
Catalase test	+	+	+	+	+	+
Oxidase test	-	-	-	-	-	-
Motility test	+	-	+	-	-	+
O/F test	F	F	F	F	O	F
Glucose (acid)	+	+	+	+	+	+

1. Bacillus = Ba 2. Staphylococcus = St 3. Enterobacteria = En
4. Corynebactrium= Co 5. Micrococcus = Mi 6. Pseudomonas = Ps

Table (2): Phytochemical components of aqueous and hexane extracts of *A. nilotica* fruit

Phytochemical compounds	Extracts	
	Aqueous	Hexane
Tannins	++++	+++
Free-anthraquinones	-	-
Co-anthraquinones	++	++
Carotenids	-	-
Proteins	+	-
Alkaloids	+	+
Flavonoids	++	+
Saponins	-	+
Reducing sugar	++	+
Carbohydrates	++	+
Terpenids	+	++
Glycosides	+	++

+ = present - = absent
++++ = high concentration
+++, ++ = moderate concentration

However, the presence of co-anthraquinones exhibited moderate concentration in both aqueous and hexane extract. The carotenoids were absent in both extract (aqueous and hexane). The protein showed its presence in the aqueous extract, but absent in hexane extract. Alkaloids and flavonoids were present in both extracts and the concentrations of flavonoids were moderate in the aqueous extract. Saponins were absent in the aqueous extract, but present in hexane extract. The reducing sugars and carbohydrates present in hexane extract and in aqueous extract with moderate concentration. Terpenoids and glycosides were present in the aqueous extract and with moderate concentration hexane extract.

Table (3) showed the effect of aqueous extract of *Acacia nilotica* on water isolate bacteria. The data showed that the different concentration of the aqueous extract showed different effects on the different test bacteria. At concentration of 25% Salmonella and Staphylococcus were highly susceptible and the Bacillus was the least susceptible. However, at concentration of 50% and 75% the most susceptible genus was Staphylococcus and the least one was Bacillus. The 100% concentration, the corynebacterium showed the highest susceptibility and the least susceptible was Bacillus the results from Table (3) showed that the most resistant genus was bacillus at all concentrations. This results was in contrast with these reported by Banso (2009) who showed that Bacillus subtilis was most susceptible to the plant extract of *Acacia nilotica* on the other hand Chandel *et al.* (1992) reported that the water extract of *Acacia nilotica* showed highest antibacterial activity against Staphylococcus, aurous, *Escherchia coli* Salmonella typhimurium, Pseudomonas aeruginosa and Klebsiella sp.

Table (4) showed the effect of different concentration of hot hexane on the test bacteria. At concentration of 25%, Salmonella was highly susceptible and the least susceptible were Bacillus, Pseudomonas and Staphylococcus. However, at 50% concentration Salmonella and Micrococcus were highly susceptible and Bacillus showed the least susceptibility. As the concentration increased to 75 and 100% the Micrococcus was highly susceptible and the Staphylococcus and Bacillus showed the least susceptibility. Similar results were obtained by Saini (2008) who reported that *Acacia*

nilotica exhibited highest activity against *Escherichia coli, Staphylococcus aureus* and *Salmonella typhi.*

Table (5) indicated that at concentration of 25, 50, 75 and 100% Salmonella was the most susceptible to the cold hexane extract.

At concentration 50, 75 and 100% Bacillus and Staphylococcus was the least susceptible to the aqueous, hot and cold hexane extract of *Acacia nilotica* pod. This may be due to the resistance of Bacillus and their ability to form resistant spores and poisonous protein capsule inside the spore.

Table (3): Effect of aqueous extract of *A. nilotica* fruit in water isolate

Treatment / Genus	Water (%)				
	25	50	75	100	Control
Salmonella	26.00^{a}	29.33^{b}	30.00^{a}	36.00^{a}	38.00^{ab}
Bacillus	21.67^{a}	22.67^{c}	25.33^{c}	28.67^{b}	40,00^{ab}
Pseudomonas	25.33^{a}	27.67^{b}	36.33^{a}	36.33^{a}	38.00^{ab}
Staphylococcus	26.00^{a}	33.33^{a}	38.67^{a}	38.33^{a}	40.00^{a}
Micrococcus	25,67^{a}	29.00^{b}	35.33^{a}	38.33^{a}	39.00^{a}
Coryne bacterium	24.33^{a}	30.67^{ab}	35.00^{a}	39.00^{a}	39.00^{b}
±SEM	1.55	1.12	1.42	1.36	2.27

Table (4): Effect of hot hexane extract of *A. nilotica* fruit in water isolate

Treatment / Genus	Hexane hot (%)				
	25	50	75	100	Control
Salmonella	26.00^{a}	28.67^{a}	30.00^{b}	33.33^{a}	38.00^{a}
Bacillus	21.33^{a}	22.33^{c}	25.00^{c}	28.00^{b}	40,00^{a}
Pseudomonas	21.33^{a}	24.33^{ab}	29.00^{b}	32.67^{a}	38.00^{a}
Staphylococcus	21.33^{a}	23.67^{bc}	24.67^{c}	27.67^{b}	40.00^{a}
Micrococcus	24,67^{a}	28.33^{a}	34.67^{a}	35.33^{a}	39.00^{a}
Coryne bacterium	22.33^{a}	26.00^{ab}	28.00^{b}	32.67^{a}	39.00^{b}
±SEM	1.72	1.47	1.04	1.43	0.85

Table (5): Effect of cold hexane extract of *A. nilotica* fruit in water isolate

Treatment / Genus	Hexane cold (%)				
	25	50	75	100	Control
Salmonella	25.33^{a}	27.67^{a}	30.00+a	32.00^{a}	38.00a
Bacillus	20.00^{b}	21.67+c	25.00^{a}	2700^{a}	40.00^{a}
Pseudomonas	20.67^{b}	23.00^{bc}	29.00^{a}	30.00^{a}	38.00^{a}
Staphylococcus	20.00^{b}	22.67^{bc}	24.00^{a}	26.67^{a}	40.00^{a}
Micrococcus	20.33^{b}	26.67+b	28.33+a	30.00^{a}	39.00^{a}
Coryne bacterium	21.00^{b}	23.00^{bc}	28.00^{a}	31.33^{a}	39.00^{a}
±SEM	0.82	1.44	2.11	2.43	0.85

(Monica, 2008). It was noticed that Salmonella, Staphylococcus, Corynebacterium and Micrococcus were more sensitive to the aqueous extract compared with hot and cold hexane extract. This may be attributed to the high concentration of tannin in the aqueous extract than in hot and cold hexane extract (Table 2).

The results also indicated that both the aqueous and hexane extracts of *Acacia nilotica* pod inhibited the growth of various genus of the test bacteria, this could be explained by that both extracts contain antimicrobial compounds like tannin, which has antimicrobial action through inhibition of protein synthesis in the bacterial cell, so resulting in killing of bacteria (Nada *et al*., 2011). Both extracts contain flavonoids which are known for their antioxidant, antimicrobial, properties (Cowan, 1999). Saponins and terpenoid have antimicrobial activities (Soetan *et al*., 2006) and glycosides also have anti-bacterial and antifungal activityand antiviral activity (Wai *et al*., 2011).

4. CONCLUSION

It can be concluded that both aqueous and hexane extracts have antibacterial activities and the aqueous extract was more effective than the hexane extract. *Acacia nilotica* fruit extract have medicinal values based on the presence of many compounds that used as antioxidant, antibacterial and antiviral activity.

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