Phytochemical and Antibacterial Screening of Aqueous andHexane Extracts of Acacia Nilotica Fruit Against Bacterial Species from Human Isolates

تقدير فعالية المستخلص المائي والهكساني لثمار السنط ضد بعض انواع البكتريا المعزولة من الإنسان

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Abstract

Aqueous and hexane extracts of *Acacia nilotica* fruit were used to evaluate the antibacterial activities against seven human isolates collected from Khartoum National Laboratory. Results indicated the presence of *Salmonella typhi*, *Shigella flexnri*, *Klelsiella pneuomoniae, Escherichia coli, Pseudomonas aernginosa, Proteus mirabilis* and *Vibrio cholerae*. Results indicated that the aqueous extract and hexane (hot and cold) extract of *Acacia nilotica* inhibited the growth of various species of Gram-negative and Gram-positive bacteria, and the aqueous extract which prepared by soaking was more effective against the bacteria than the hot hexane extract.Wherease the extraction method affect on the efficiency extract. Also the phytochemical analysis results revealed the presence of tannins, co-anthraquinones, alkaloids, flavonoids, saponin, terpenoids and glycosides, the objectives of the study were to evaluate the phytochemical constituents of the aqueous and hexane extracts of *Acacia nilotica* fruit, and to assess the antibacterial activity of these constituents. The experimental and analytical method was used. The importance of the study was derived from that many diseases were spread among inhabitants in rural areas, and the resistance of the bacteria to certain antibiotics. This inforced scientists to look for other alternative, that the using of the aromatic and medicinal plants. As the components of these plants were safe, effective and cheap.

Keywords: Antibacterial activity, *Acacia nilotica,* phytochemical screening, antibiotics resistance, human isolates.

المستخلص

أستخدم المستخلص المائي والهكسان لثمار شجرة السنط بهدف تقدير فعالية هذه المستخلصات ضد نشاط سبعة عزلات من البكتريا الممرضة للإنسان أُخذت من المعمل القومي في الخرطوم والتي أظهرت وجود سالمونيلا تيفي، شيجلا فلكسنري، كليبسلا بنومينا، إيشرشيا كولاي، بسودوموناس ارجينوسا، بروتيس ماربليس، فيبريوكوليرا. أظهرت النتائج أن المستخلص المائي والهكسان (ساخن، بارد) له تأثير إيجابي ضد نمو البكتريا الموجبة والسالبة لصبغة جرام وثبت أن المستخلص المائي ألمحضربالتنقيع اكثر فعالية من مستخلص الهكسان الحار.حيث ان طريقة الاستخلاص تؤثر علي فعالية المستخلص. كما أظهرت نتائج التحليل الكيميائي احتواء المستخلصات على المواد الفعالة الموجودة في النباتات الطبية مثل: التانين، القلويد، الفلافنويد، الصابونين، التربونيد، الجلايكوسيد. تمثلت أهداف الدراسة في معرفة المواد الكيميائية التي تحتويها ثمار السنط ومعرفة تأثيرها على الميكروبات المعزولة من الإنسان. أُستخدمت الطريقة التجريبية التحليلية في جمع وتحليل العينات. نبعت أهمية الدراسة من انتشار الأمراض خاصة وسط سكان الريف ومقاومه البكتريا للمضادات الحيوية .إضافة للاتجاه الحديث للنباتات الطبية والعطرية كمصدر للأدوية آمن فعال وقليل التكلفة كبديل للمضادات الحيوية.

كلمات مفتاحية:

نشاط مضاد للبكتيريا،أكاسيا نيلوتيكا،الفحص الكيميائي ، مقاومة للمضادات الحيوية، عزلات الإنسان .

Introduction

Plants have great significance due to their nutritive value and are also a major source of medicines. Food plants, including fruits, vegetable and species are primary source of naturally occurring nutrients essential for human health [1].

Medicinal plants constitute the group of plants mainly used for health care. Medicinal plants are claimed to possess antibiotic properties and are used extensively by the tribal people worldwide [2], studies indicated that herbs have medicinal property due to presence of different active principles like alkaloids, volatile essential oils, glycosides, resins, oleoresins, steroids, tannins, terpenes and phenols[3]. The World Health Organization (WHO)supports the use of medicinal plant, provided it is proven to be efficacious and safe[4].

Acacia nilotica is known asamulti-purpose medicine, used to treat specific ailments. *Acacia* is apomtropical and subtropical genus with species abundant throughout Asia, Australia, Africa and America[5]. Studies have reported that pod extract of *Acacia nilotica* showed antibiotic activity against some bacterial species [6][7] [8].

The objective of this study was to evaluate the phytochemical constituents of the aqueous and hexane (hot and cold) fruit extracts of *Acacias nilotica* and to assess the antibacterial activity of these extracts against some strains of microorganisms found in human isolates.

Materials and Methods

Collection of plant material

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

Collections of human isolates:

Human isolates were collected from the National Laboratory(Staak).The isolates were undergone some biochemical tests and the identification of the samples was done according[9].The extraction was done according to [10].

Preparation of aqueous extract:

Preparation of aqueous extract, hot and cold hexane extract were carried out according [10].

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 was extracted. Then the extract was filtered on Whatman's No. 1 filter paper, transferred into sterile bottle and stored in a refrigerator at 4°C until used[10].

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°C until used[10].

Preparation of cold hexane extract:

100g of the powdered sample was weighted and replaced in sterile bottle, 150ml of hexane 95% was added and shake well from time to time. After 72 hours the extract was filtered on Whatmans No. 1 filter paper, then transferred into sterile bottle and stored in a refrigerator at 4°C[10].

Preparation of concentration extracts:

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

Antimicrobial Screening:

The antibacterial activity of the plant extracts were determined according to [11]. Using agar well diffusion method. The bacteria isolates collected in slant of nutrients agar sub-cultured into prepared nutrients broth and incubated at 37˚C for 24h and standardized to 0.5 mc-farland scales (10^8 CFU/ml) in a prepared normal saline. Into prepared nutrient agar each plate was inoculated with bacteria suspension using a sterile loop. Four wells were made in the plates using a sterile tip (7mm) indiometer. Each of the aqueous and hexane concentration extracts were transferred in to the wells with micropipette then allowed to stand for 30 minutes at room temperature for proper diffusion. The plates incubated at 37˚C for 24h. A control were setup in parallel. After 24h clear zone of inhibition were measured and compared with that of the standard control. For per strains the assay was triple replicated and took the mean. The results were analyzed and means were compared using least significant differences (LSD).

Phytochemical screening:

The phytochemical screening tests were carried out on the aqueous extract and hexane extract using standard methods to identify the constituents as described by[12].

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 heat. 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. Abluish-black or brownish green precipitate indicated the presence of tannins.

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.

Test for terpennoids:

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.

Test for saponins:

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

Test for alkaloids:

1g of powdered sample was boiled with 10ml of dilute hydrochloric 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.

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 sulphuric acid. Abrown ring at the interface indicated the deoxy sugar characteristics of cardenolides. Avoilet ring may appear below the ring, while in the acetic acid layer a greenish ring may be formed.

Test for co-anthraquinones:

1g of the powder 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.

Test of free anthraquinones:

5ml of chloroform was added to 0.5g of the powdered sample, then the resulting mixture was shaken for 5 minutes, then filtered. The filtrate was shaken with equal volume of 10% ammonia solution. The presence of a bright pink colour in the aqueous layer indicated the presence of free anthraquinones.

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.

Test for protein:

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.

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

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 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.

Results and Discussion

The results from Table (1) revealed the presence of *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella typhi, Shigella flexneri, Proteus mirabilis, Klebsiella pneumoniae* and *Vibrio cholerae* from human isolates.

The phytochemical analysis of *Acacia nilotica* fruit showed the presence of various phytoconstituents including tannin, co-anthraquinones, alkaloids, saponins, flavonoids, carbohydrates, terpenoids, glycosides and carotonoids. In this respect the phytochemical investigation of *Acacia nilotica* fruit revealed the presence of Carbohydrates, saponins, glycosides and flavonoids [13].

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 showed the absence of free anthraquinones in both aqueous and hexane extract, but the presence of co-anthraquinones exhibited moderate concentration in both aqueous and hexane extracts. The carotenoids were absent in both extracts (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 concentration of flavonoids were moderate (++) in the aqueous extract saponins were absent in the aqueous extract, but present in hexane extract. The reducing sugar and carbohdyrates represent in both extracts but the concentration in the aqueous extract was moderate (++). Terpenoids and glycosides were present in the aqueous extract and in the hexane extract with moderate concentration (++)

No.	Species	Lact	Man	Glu	Suc	OX	Cit	MIU medium			KIA medium			
								Mot	Ind	Urea	Slope	Butt	H_2S	Gas
1.	*E. coli*	+	+	+	D	-ve	-ve	+ve	+ve	-ve	Y	Y	-ve	+ve
2.	Pseudomonas aeruginosa	-	-	d	-	+ve	+ve	+ve	-ve	+ve	R	R	-ve	-ve
3.	Salmonealla typhi	-	+	+	-	-ve	-ve	+ve	-ve	-ve	R	Y	+ve	-ve
4.	Shigella flexneri	-	d	+	-	-ve	-ve	-ve	-ve	-ve	R	Y	-ve	-ve
5.	Proteus mirabilis	-	-	+	d	-ve	+ve	+ve	-ve	+ve	R	Y	+ve	+ve
6.	Klebsiella pneumoniae	+	+	+	+	-ve	+ve	-ve	-ve	+ve	Y	Y	-ve	+ve
7.	Vibrio cholerae	-	+	+	+	+	d	+ve	+ve	-ve	R	Y	-ve	-ve

Lact = lactose
Man = mannitol
Glu = glucose
Suc = sucrose
OX = oxidase test
Cit = citrate test
d = different strains give different results

Mot = motility test
Ind = Indol test
Urea= ureaset test
H_2S = hydrogen sulphide
R = red (alkaline reaction)
Y = yellow (acid reaction

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

Phytochemical compounds	Aqueous extract	Hexane extract
Tannins	++++	+++
Free-anthraquinones	-	-
Co-anthroquinones	++	++
Carotenoids	-	-
Proteins	+	-
Alkaloids	+	+
Flavonoids	++	+
Saponins	-	+
Reducing sugars	++	+
Carbohydrates	++	+
Terpenids	+	++
Glycosides	+	++

+ = Present, - = absent
++++ = High concentration +++, ++ = moderate concentration

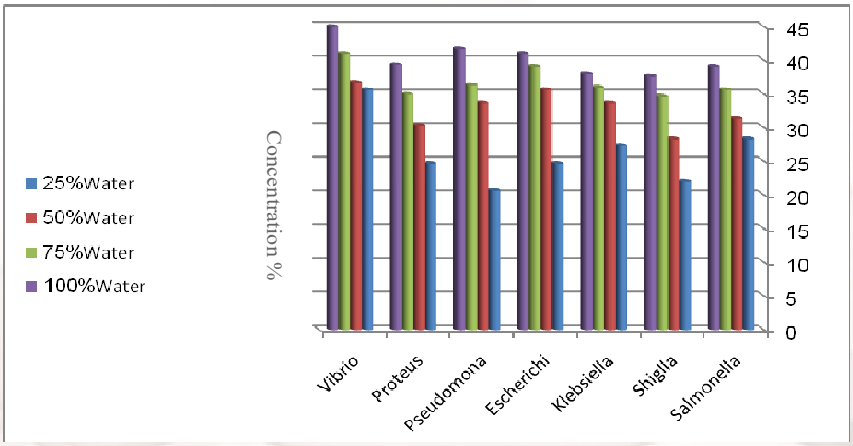
Table (3) indicated that the most susceptible bacteria at different concentration
(25, 50, 75, 100%) of the aqueous extract was *Vibrio cholerae*. On the other hand the least susceptible bacteria at concentration 25% was *Pseudomonas aeruginosa*, but at 50, 75 and 100% concentration *Shigella flexneri* was the least susceptible bacteria. As it can be seen from the results (Table 3) the aqueous extract showed inhibition at lower concentration 25% against all test bacteria of human isolate even on *pseudomonas*, which is resistant to most of the commonly used antibiotics[9]. In connection to this[7] reported that *Acacia nilotica* extract exhibited highest activity against three bacterial strains (*Escherichia coli, Staphylococcus aureus*and *Salmonella typhi.* In(Table 3) the statistical analysis showed in $p \leq 0.05$ that there was a significant difference between the different isolates using the different concentration (25,50,75,100%).

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

Treatment / Genus	Water (%)			
	25	50	75	100
Salmonella typhi	28.23^{b}	31.33^{bc}	35.67^{b}	39.00^{cb}
Shigella flexneri	22.00^{c}	28.33^{c}	34.67^{b}	37.67^{d}
Klebsiella pneumoniae	27.33^{b}	33.67^{ab}	36.00^{b}	38.00^{d}
Escherichia coli	24.67^{bc}	35.67^{a}	39.00^{a}	41.00^{bc}
Pseudomona aerguinosa	20.67^{c}	33.67^{ab}	36.33^{b}	41.67^{b}
Proteus mirabilis	24.67^{bc}	30.33^{c}	35.00^{b}	39.33^{bc}
Vibrio cholerae	35.67^{a}	36.67^{a}	41.00^{a}	45.00^{a}
±SEM	1.65	1.49	0.72	0.72

- (LSD)by letters (a,b,c,d)

Figure(3): Effect of Aqueous extract of *A.nilotica* Fruit in Human Isolate

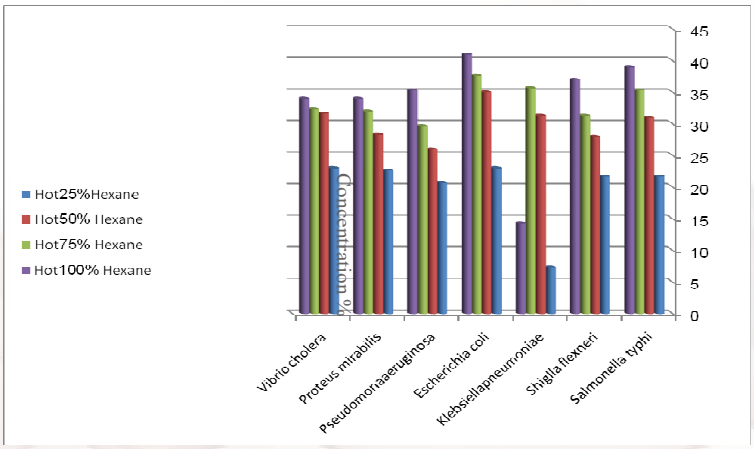


The hot hexane extract (Table 4) with concentration 25% showed that *Escherichia coli* and *Vibrio cholerae* were the most susceptible bacteria and the least susceptible was *Klebsiella pneumoniae*. However, at concentration 50 and 75% the most susceptible bacteria was *Escherichia coli* and the least one was *Pseudomonas aeruginosa*. As the concentration reached 100%, *Escherichia coli* was the most susceptible and the least one was *Klebsiella pneumonia*. In(Table 4) the statistical analysis showed in $p \leq 0.05$ that there was no significant difference between the isolates in concentration(25%) except in *Klebsiella pneumonia* which showed the least susceptible. But in concentration(50,75,100%) there was significant difference between the different isolates.

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

Treatment / Genus	Hexane hot (%)			
	25	50	75	100
Salmonella typhi	21.67^{a}	31.00^{b}	35.33^{ab}	39.00^{a}
Shigella flexneri	21.67^{a}	28.00^{bc}	31.33^{bc}	37.00^{a}
Klebsiella pneumoniae	7.33^{d}	31.33^{ab}	35.67^{ab}	14.33^{b}
Escherichia coli	23.00^{a}	35.00^{a}	37.67^{a}	41.00^{a}
Pseudomona aerguinosa	20.67^{a}	26.00^{c}	29.67^{c}	35.33^{a}
Proteus mirabilis	22.67^{a}	28.33^{bc}	32.00^{bc}	34.00^{a}
Vibrio cholerae	23.00^{a}	31.67^{ab}	32.33^{bc}	34.00^{a}
±SEM	2.21	1.22	1.89	3.15

Figure (4) :Effect of Hot Hexane extract of *A.nilotica* Fruit in Human Isolate.

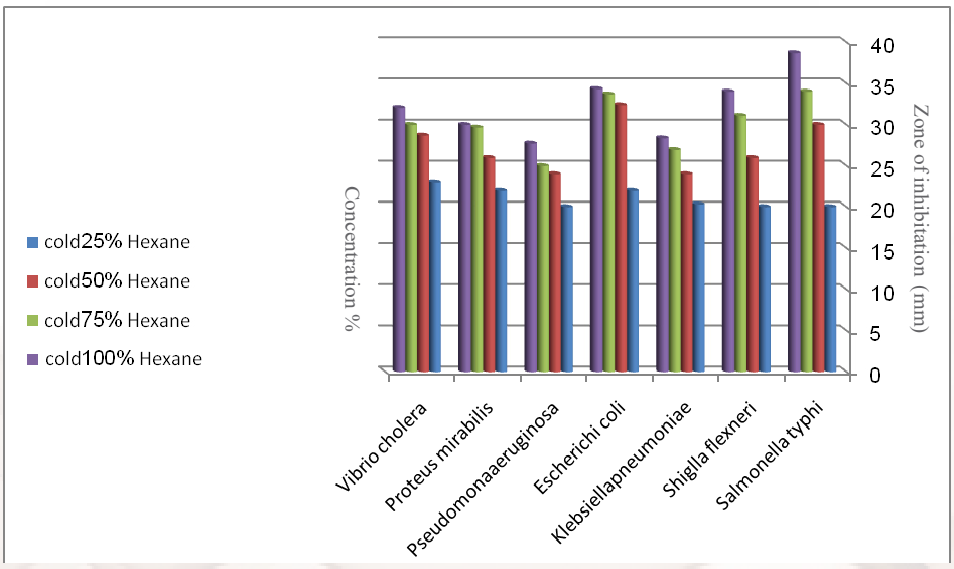


The cold hexane extract (Table 5) showed that *Vibrio cholerae* was most susceptible at 25% concentration. At concentration 50% *Escherichia coli* was the most susceptible and the most resistant were *Klebsiell pneumoniae* and *Pseudomonas aureginosa.* As the concentration reached 75 and 100% *Salmonella typhi* was the most sensitive and the resistant bacteria was *Pseudomonas aeruginosa*. In(Table 5) the statistical analysis showed in $p \leq 0.05$ that there was a significant difference between the different isolates using the different concentration (25,50,75,100%).

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

Treatment / Genus	Hexane cold (%)			
	25	50	75	100
Salmonella typhi	20.00^{b}	30.00^{ab}	34.00^{a}	38.67^{a}
Shigella flexneri	20.00^{b}	26.00^{bc}	31.00^{cd}	34.00^{b}
Klebsiella pneumoniae	20.33^{b}	24.00^{c}	27.00^{bc}	28.33^{cd}
Escherichia coli	22.00^{ab}	32.33^{a}	33.67^{a}	34.33^{b}
Pseudomona aerguinosa	20.00^{b}	24.00^{c}	25.00^{d}	27.67^{d}
Proteus mirabilis	22.00^{ab}	26.00^{bc}	29.67^{bc}	30.00^{cd}
Vibrio cholerae	23.00^{a}	28.67^{abc}	30.00^{bc}	32.00^{bc}
±SEM	0.83	1.73	1.19	1.24

Figure (5): Effect of Cold Hexane extract of *Acacia. nilotica* Fruit in Human Isolate



The results of this study showed that the aqueous and hexane extract of *Acacia nilotica* fruit inhibited the growth of various species of Gram-negative and Gram-positive bacteria. These results were in agreement with the result obtained by[14], but was in contrast with the results obtained by[15] who reported that both extracts were highly inhibitory to Gram-positive in comparison with Gram-negative bacteria, because of the different in cell wall composition of the bacteria. The results of this study may be attributed to the high concentration of tannins and the other antimicrobial compounds. Although Gram-negative posses two cytoplasmic membrane which contains toxic lip polysacharide (LPS) in which protect and inhibit the antibiotic for reaching the peptidoglycan, but tannins are well known for their ability to damage the phospholipids and the lipoproteins and the lipopolysaccharide which ingredient the outer cytoplasmic membrane[16].

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 activity.

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