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RESEARCH TITLE

Extraction and Biological Activity of the Fixed oils From Some Sudanese *Acacia* Species

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Abstract

Recently, there has been a lot of attention focused on producing medicines and other natural products from the plant. The paper research aims to study antimicrobial, antioxidant activity for seeds oils of three species from Sudanese *Acacia* plants *(Acacia seiberana var villosa, Acacia Seyal var fistula, Acacia polyacantha*). Antioxidant activity was determined by DPPH assay. The disc diffusion method was used to determine the antimicrobial activity of oils against five standard human pathogens Gram-positive *(Staphylococcus aureus (ATCC25923*), *Bacillus Subtilis (NCTC8236*), Gram-negative (*Esherichia coil (ATCC25922)* and pseudomonas aerugihosa *(ATCC2785*) and fungi *candida albicans.* The oils showed different antimicrobial responses against organisms. With the typical antibiotic, the result was compared. The oils from three *Acacia* species at the concentration (12.5 -100) mg/ml are exhibited inactive against *Esherichia coli, staphylococcus auras* and *candida albicans*. *seiberana var villosa* oil and *Acacia Seyal var fistula* oil It also showed partially activity against *pseudomonas arugionsa* and *Bacillus subtilis* at the concentration (12.5 - 100) mg/ml. *Acacia polyacantha* oil exhibited partially activity against *Bacillus subtilis* and *pseudomonas arugionsa* at the concentration (12.5-100) mg/ml, (12.5-25) mg/ml, respectively and active at the concentration (50-100) mg/ml against *pseudomonas aeruginosa*. *Acacia seiberana var villosa Acacia polyacantha* oils showed that moderate antioxidant activity (58, 60) % respectively. *Acacia Seyal* var *fistula* oil showed that low antioxidant activity 21%.

Key Words: Extraction, Biological Activity, Acacia, Fixed Oil

عنوان البحث

الاستخلاص والفعالية الحيوية للزيوت الثابتة لبعض نباتات الاكاشيا السودانية

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المستخلص

في الاونة الاخيرة كان الكثير من الاهتمام الذي يرتكز علي انتاج الادوية والمنتجات الطبيعية الاخرى من النبات .تهدف الورقة البحثية الي دراسة الفعالية المضادة للمكروبات ومضادات الاكسدة بذور ثلاثة انواع من الاكاشيا السودانية(الكوك ,الصفير ,الكاكموت) تم استخدام طريقة انتشار القرص لتحديد النشاط المضاد للميكروبات للزيوت ضد خمسة انواع مسببات الأمراض البشرية تتمثل في بكتريا جرام الموجبة وتشمل (العصوية الرقيقة والمكورات العنقودية) وبكتريا جرام السالبة وتشمل (الاشريكيةالقولونية والزائفة الزنجارية) وفطر الكانديدا اظهرت الزيوت استجابات مختلفة لمضادات الميكروبات ضد الكائنات الحية تمت مقارنة النتائج بالمضادات الحيوية القياسية اظهرت جميع الزيوت انها غير فعال ضد الاشريكية القولونية والمكورات العنقودية وفطر الكانديدا كما اظهرزيت الكوك وزيت الصفير فعالية جزئية ضد الزائفة الزنجارية والعصوية الرقيقة عند تركيز (12.5 – 100) مجم/مل , اظهر زيت الكاكموت انه غير فعال ضد العصوية الرقيقة(12.5 – 100) مجم /مل والزائفة الزنجارية بتركيز (12.5–25) مجم/مل كما اظهر الزيت فعالية ضد الزائفة الزنجارية بتركيز(50–100) مجم / مل .اظهرت هذة الزيوت زيت الكوك وزيت الكاكموت فاعلية معتدلة كمضاد للأكسدة(60, 58)% علي التوالي . اظهر زيت الصفير فعالية منخفضة كمضاد للأكسدة 21%.

الكلمات المفتاحية: استخلاص , الفعالية الحيوية , الاكاشيا , الزيوت الثابتة

Introduction

Chemical elements and substances derived from plants, animals, and minerals are considered natural products. They served as the foundation for all previous conventional medical systems used throughout the world. As food and medicine, they employ plants, their parts, and the oils made from them. [1]Natural goods, including medicinal plants, are increasingly used in basic healthcare globally, particularly in underdeveloped nations. Numerous studies are conducted to find novel natural product alternatives that can treat human disorders including diabetes and cardiovascular disease. [2] Due to their perceived safety over synthetic chemical compounds, customers' interest in natural products has increased over the past few years. As a result, goods like essential oils have become quite popular in the food and cosmetics industries.[3] Sudan is located in tropical Africa and has a diverse population as well as a high plant diversity. Due to cultural and economic factors, traditional medicine plays a significant role in Sudan and other developing nations.[4]the widespread use of medicinal plants for the treatment of various diseases has motivated many researchers to examine their biological activities[5, 6]. Additionally, natural ingredients can help in the search for new antioxidant [7] and antibacterial [8] components.

Greek for "thorns," Acacia is the largest genus of shrubs and trees in the pea family Fabaceae subfamily Mimosoideae, with about 1200 species primarily found in tropical and subtropical regions.[9] Species of Acacia can flourish under adverse conditions.[10,11]

In Sudan, acacia species (31 species) prevail and are highly significant due to their medical and commercial value in addition to the gum that some of them generate. They make up around one-third of all African species. [12]

1. Plant material:-

The seeds of Sudanese (*Acacia seiberana* var *villosa*, *Acacia seyal* var *fistula*, *Acacia polyacantha*) were collected from Sennar (AL dinder), AL Gazira (AL faw village), Blue Nile) respectively for the forest Research Center, Khartoum Sudan.

2. Instruments

DPPH radical scavenging was calculated using a modified version of Shimada et al (1992) approach.

1.2Test organisms

Acacia seed oils were screened for antibacterial and antifungal activities using the standard microorganisms shown in table (1).

Table (1): Test organisms.

S. No	Microorganism	Type
	Bacillus subtilis	G+ve
	Staphylococcus aureus	G+ve
	Pseudomonas aeroginosa	G-ve
	Escherichia coli	G-ve
	Candida albicans	fungus

2. Methods:-

2.1 Extraction of oils from *Acacia* species:-

500g of dry-powdered *Acacia* seeds were macerated in n-hexane for 48 hours at room temperature. Under reduced pressure, the solvent was withdrawn, and the oils were preserved at 4°C in the refrigerator for later manipulation.

2.2 Antimicrobial assay:-

2.2.1 Preparation of bacterial suspensions:-

The test organisms' 24-hour broth culture was divided into one-ml aliquots and aseptically dispensed onto nutrient agar slopes before being incubated for 24 hours at 37°C. A suspension containing around 108–109 colony- forming units per ml was created by harvesting the bacterial growth, washing it off with sterile normal saline, and then suspending it in 100 ml of normal saline. Until it was used, the suspension was kept at 4°C in the refrigerator. Using the surface viable counting approach, the average number of viable organisms per ml of the stock suspension was calculated. Serial dilutions of the stock suspension were produced in sterile normal saline in tubes, and the relevant dilutions were applied to the surface with one drop quantities (0.02 ml) using an adjustable capacity micropipette.[12]

2.2.2 Preparation of fungal suspensions:-

On potato dextrose agar, fungi were kept alive for four days while incubated at 25°C. The fungus was removed, cleaned with sterile normal saline, and the suspension was kept in the fridge until it was needed[12].

2.2.3 Testing for antibacterial activity:-

The cup-plate agar diffusion method was adopted, with some minor modifications, to assess the antibacterial activity. 200 ml of sterile, molten nutrient agar was combined with 2 ml of the standardized bacterial stock suspension and kept at 45 °C in a water bath. (20 ml) Aliquots of the nutrient agar that had been incubated were put into sterile Petri dishes. It was let to sit for a while. These plates were split into two pieces. Using a sterile cork borer (No. 4), two cups in each half (10 mm in diameter) were cut; each half was intended for one of the test samples. Separate Petri dishes were designed for standard antimicrobial chemotherapeutics. The agar discs were removed and alternate cups were filled with (0.1 ml) samples of each test solution using adjustable volume micro titer pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours. The aforementioned process was performed with test solutions and standard chemotherapeutics at various concentrations. After incubation, the diameters of the resultant growth inhibition zones were measured in triplicates and averaged.[12]

2.2.4 Testing for antioxidant activity:-

At the Medicinal and Aromatic Plants Research Center, the experiment was carried out. National Center for Research Institute, Khartoum, Sudan. According to the approach .The DPPH radical scavenging was calculated.

Shimada et al. (1992), with a few changes. Test samples are placed in a 96-well plate. Were given 30 minutes to react with 2,2Di -1-picryl-hydrazine (DPPH) at 37C°. The

level of DPPH was maintained at (0.3 mm). They were the test samples DMSO was used to dissolve it while ethanol was used to prepare DPPH. Using a multi-plate, the decrease in absorbance was measured after incubation. Using a multi -plate, the decrease in absorbance was measured after incubation. Spectrophotometer reader Samples' percentage of radical scavenging activity was compared to a control group that received DMSO treatment to determine. Every test and Analysis was carried out three times.

$$\text{Radical scavenging activity\%} = \frac{(\text{absorbance of control} - \text{absorbance of sample})}{\text{Absorbance of control}} \times 100$$

3. RESULTS AND DISCUSSION:-

In this study, three Sudanese acacia species (*Acacia seiberana* var *villosa*, *Acacia seyal* var *fistula* and *Acacia polyacantha*) have been assessed for their antimicrobial and antioxidant activity.

3.1 Antimicrobial activity:-

In cup plate agar diffusion assay, the oils was screened for antimicrobial activity against five standard human pathogens. The average of diameters of the growth of inhibition zones is depicted in Table (2). The results were interpreted in terms of the commonly used terms (>9mm: inactive; 9-12mm: partially active; 13-18mm: active ;< 18mm: very active. The antimicrobial efficacy of common antibacterial and antifungal chemotherapeutic drugs against common bacteria and fungi, respectively, is shown in Tables (3) and (4).

3.1.1 *Acacia seiberana var villosa*

Acacia seiberana var *villosa* oil exhibited inactive against *Esherichia coli*, *staphylococcus auras* and *candida albicans* at the concentration (12.5 -100) mg/ml and partially activity against *pseudomonas arugionsa* and *Baillus subtilis* are shown in table (2)

Table (2) Antimicrobial activity of *Acacia seiberana* var *villosa* seed oil:

Oil	**Concentration**	***EC***	***Ps***	***Sa***	*Bs*	***Ca***
Acacia seiberana var *villosa*	100	-	11	-	11	-
	50	-	11	-	10	-
	25	-	11	-	10	-
	12.5	-	12	-	9	-

>18 = very active

(-) means = inactive

Table (3) Standard antibacterial activity: MDIZ (mm).

Drug	**Conc. mg/ml**	***Bs.***	***Sa***	***Ec.***	***Ps.***
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	**11**	**15**	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table (4) Standard antifungal effects on common fungi:

M.D.I.Z. (mm)

Drug	**Conc. mg/ml**	***Ca.***
Clotrimazole	30	38
	15	31
	7.5	29

Sa.: Staphylococcus aureus.

Ec.: Escherichia coli.

Pa.: Pseudomonas aeruginosa.

Bs.: Bacillus subtilis.

Ca.: Candida albicans.

M.D.I.Z: Mean diameter or growth inhibition zone (mm).

1.3.1.1 Antioxidant activity of *Acacia seiberana* var *villosa* seed oil

Acacia seiberana var *villosa* oil showed that moderate antioxidant activity 58% shown in table (5).

Table (5) Antioxidant activity of *Acacia seiberana* var *villosa* seed oil:-

No	**Sample**	**%RSA±SD (DPPH) µg/mL**
1	Oil	58±0.02
2	Propyl Gallate (Standard)	91±0.01

3.1.2 *Acacia seyal* var *fistula*

Acacia Seyal var *fistula* oil exhibited inactive against *Escherichia coli*, *staphylococcus auras* and *candida albicans* at the concentration (12.5 -100) mg/ml. It also showed partially activity against *pseudomonas arugionsa* and *Bacillus subtilis* are shown in table (6)

Table (6) Antimicrobial activity of *Acacia seyal* var *fistula* seed oil

Oil	**Concentration**	***EC***	***Ps***	***Sa***	*Bs*	***Ca***
Acacia seyal var *fistula*	100	-	12	-	10	-
	50	-	11	-	10	-
	25	-	11	-	10	-
	12.5	-	10	-	9	-

3.1.2.1 Antioxidant activity of *Acacia seyal* var *fistula* seed oil:

The result show that low antioxidant activity of *Acacia seyal* var *fistula* seed oil was 21% shwon in table (7).

Table (7) Antioxidant activity of *Acacia seyal* var *fistula* seed oil:-

No	**Sample**	**%RSA±SD (DPPH) µg/mL**
1	Oil	21±0.01
2	Propyl Gallate (Standard)	91±0.01

3.1.3 *Acacia polyacantha*

Acacia polyacantha oil exhibited inactive against *Escherichia coli*, *staphylococcus auras* and *candida albicans*.at the concentration (12.5-100) mg/ml partially activity against *Bacillus subtilis* and *pseudomonas aeruginosa at* the concentration (12.5-100) mg/ml, (12.5-25) mg/ml respectively , and active at the concentration (50-100) mg/ml against *pseudomonas aeruginosa are* shown in table (8).

Table (8) Antimicrobial activity of *Acacia polyacantha* seed oil:

Oil	**Concentration**	***EC***	***Ps***	***Sa***	*Bs*	***Ca***
Acacia polyacantha	100	-	13	-	12	-
	50	-	13	-	11	-
	25	-	11	-	11	-
	12.5	-	9	-	10	-

1.3.3.1 Antioxidant activity of *Acacia polyacantha* seed oil:

The oil showed that moderate antioxidant activity 60%.

Table (9) Antioxidant activity of *Acacia polyacantha* seed oil:-

No	**Sample**	**%RSA±SD (DPPH) µg/mL**
1	Oil	60±0.08
2	Propyl Gallate (Standard)	91±0.01

Conclusion:-

From the result finding of the present investigation it can conclude that:

1- *Acacia seiberana* var *villosa* , *Acacia seyal var fistula* and *Acacia polyacantha* oils the exhibited inactive against *Esherichia coli*, *staphylococcus auras* and *candida* at the concentration (12.5 -100) mg/ml. It also showed partially activity against *pseudomonas arugionosa* and *Bucillus subtilis* for *Acacia seiberana* var *villosa*, *Acacia seyal* var *fistula* at the concentration (12.5-100) mg/ml and *Acacia polyacantha* fixed oil showed partially activity against at *Bucillus subtilis* and *pseudomonas arugionosa* the concentration (12.5-100) mg/ml, (12.5-25) mg/ml, respectively and active at the Concentration (50-100) mg/ml against *pseudomonas aeruginosa*.

2- *Acacia seiberana* var *villosa*, *Acacia polyacantha* oils showed that moderate antioxidant (58%, 60 %) respectively and *Acacia seyal* var *fistula* oil showed that low antioxidant (21%).

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