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Phytochemical Screening and Biological Activity of *Balanites aegyptiaca* Stem Bark

Isam Eldin Fahmi Amin Mutwali and Suad Abdelgadir

Faculty of Education, Department of Chemistry, University of Al-Zaiem Al-Azhari, Khartoum, Sudan

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ABSTRACT

Balanite aegyptiaca is an important woody, true xerophytic tree of tremendous importance. The plant has been used in a variety of folk medicines, e.g. an anthelminthic activity against hepatic worms (Schistosoma mansoni and Fasciola gigantica), in addition to purgative, febrifuge etc. The aim of the study was to find out the nature of bioactive chemical constituents, and to determine their bioactivity such as antibacterial and antifungal activity of Balanites aegyptiaca stem bark. Phytochemical screening of the plant stem bark was carried out according to standard method of Harbone[1]. While the microbial activities were done by following disc diffusion assay. A qualitative Phytochemical analysis was performed for the detection of secondary metabolisms, which indicates the presence of Alkaloids, Tannins, Triterpenoids, Saponins. Also appositive test indicates the presence of two biomolecules Amino Acids and Carbohydrates. The microbial activities were provide that most of the extracts methanol, ethyl acetate, chloroform and aqueous extract showed antibacterial activities with high to moderate inhibitory effect (25 mm , 23 mm for Escherichia coli and Klebsiella pneumoniae respectively). Only the chloroform and the aqueous extracts of the plant organ showed positive antifungal activity (15 and 14 mm respectively), while other extracts methanol and ethyl acetate showed negative results.

Key words: Balanites aegyptiaca, Phytochemical Screening, Plant Extracts, Antibacterial, Antifungal Activity

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INTRODUCTION

Balanites aegyptiaca is a widely grown desert plant with multi-use potential. It is mainly found in arid and semi–arid areas throughout Africa, the Middle East, and South Asia. It is believed that the plant is indigenous to all dry lands south of the Sahara, extending southward to Malawi in the Rift Valley, and to the Arabian Peninsula. It has wide ecological distribution, but it is mainly found on level alluvial sites with deep sandy loam and free access to water. It is a lowland species, growing up to 1000 m altitude in areas with mean annual temperature of 20 to 30°C and mean annual rainfall of 250 to 400 mm [2]. In Sudan it is widely spread in the northern arid and the central semi-arid regions [3].

1.1. Traditional Uses of The Plant

In many African countries (e.g. Senegal, Nigeria, Ethiopia and Sudan) *Balanites aegyptiaca* has been reported to be an anti-helminthic, a purgative, febrifuge, emetic and can also cure other types of ailments like skin boils, malaria, wounds, colds, syphilis, liver and spleen disorders[4]. Various parts of this plant have their own traditional medicinal properties. The seed is used as a febrifuge [5], and its oil is used to treat tumors and wounds [6]. An aqueous extract of the bark is used in Sudanese folk medicine in the treatment of jaundice [7, 8] . The kernel oil exhibited anticancer activity against lung, liver, and brain

carcinoma cell lines. It also has anti-mutagenic activity against *Fasciola gigantica*-induced mutagenicity besides anthelmintic activity against hepatic worms (*Schistosoma mansoni*), the aqueous leaf extract and saponins isolated from its kernel cakes have antibacterial activity [9,10]. The branches are used as tooth brush [11]. The root extracts have proved to be slightly effective against experimental malaria [12].

1.2. Chemical Constituents of The Bark

The bark is reported to contain alkaloids namely, N-trans-feruloyltyramine and N-cis-feruloyltyramine, [13], and a new sugar, diglucosyldirhamnoside [14,15, 16]. Several previous phytochemical studies [17,18, 19] were detected the presence of Tannins, Triterpenoids and Saponins in this part of the plant. Saponins compounds were detected in all the other plant organs [20, 21].

1.3. Pharmacological Properties of *B. aegyptiaca*

The pharmacological activity of the plant is attributed to its chemical constituents. Several studies confirmed that the furanocoumarin-bergapten isolated from the stem bark of *Balanite aegyptiaca* has a wide range of biological activity, and are used to treat dermatological diseases [22,23, 24]. The stem bark of *B. aegyptiaca* exhibited cytotoxic activity, significant effectiveness and has the potential for treatment of obstructive jaundice in human [25]. Acetone and methanolic extracts of stem bark protect Wistar albino rats against viper venom at lethal dose (0.194 mg/mL), when administered intramuscularly[7].

EXPERIMENTAL SECTION

2.1. plant material

The plant materials were collected from Gazira state (central Sudan) during summer season (August and September), 2014. The plant species was identified at the herbarium of the Department of Botany, University of Khartoum, Sudan.

2.2. Processing and Extraction of Plant Samples

The dried powdered bark (200 g) was extracted successively by ethyl acetate, chloroform and ethanol in a Soxhlet extractor by continuous hot percolation. Each time before extracting with the next solvent of higher polarity the powdered material was dried in a hot air oven below 50 ºC for 10 minutes and dried in hot air oven [1].

2.2. 1. Test for Alkaloids

2.5 g of plant material was dissolved in 20 ml methanol. The methanolic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2N HCl (10 ml). After cooling, the mixture was filtered and the filtrate was divided into two equal portions. One portion was treated with a few drops of Mayer's reagent and the other with equal amounts of Wagner's reagent. A slight turbidity or heavy precipitate in either one or the two test tubes was taken as presumptive evidence for the presence of alkaloids [1].

2.2.2. Test for Flavonoids [27]

1 g of plant material was dissolved in 20 ml methanol. The methanolic was evaporated to dryness and was defatted by washing several times with petroleum ether. The defatted residue was dissolved in 10 ml ethanol. To check for the presence of flavonoids the filtrate was tested as follows:

A. To 2 ml of the filtrate 0.5 ml of conc. HCl and a few magnesium turnings was added. Color changes to pink or red indicate a presumptive evidence of the presence of flavonoids
B. To 3 ml of the filtrate 4ml of 1% KOH was added. A dark yellow color indicates the presence of flavonoids compounds.
C. To 3 ml of the filtrate in a test tube, 4 ml 1 % $AlCl_3$ in methanol was added. Formation of a yellow color indicates the presence of flavonols, flavones and chalcones.

2.2.3. Test for Tannins

0.5 g of the dried powdered sample of the stem bark was boiled in 20 ml of water in a test tube and then filtered. A few drops of 1 % ferric chloride was added. A brownish green or blue-black coloration indicate the presence of tannin [26].

2.2. 4. Test for Saponins

2 g of the powdered sample of the stem bark was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and was shaken vigorously. A formation of an emulsion indicates the presence of saponins [28].

2.2. 5. Test for Glycosides

A. **Keller Killiani Test** (Cardiac glycosides)

0.5 g of the plant extract was added to 0.4 ml glacial acetic acid containing a trace amount of ferric chloride solution and then a 0.5 ml of concentrated sulphuric acid was carefully added along the sides of the test tube. Appearance of a blue colour in the acetic acid layer will indicate the presence of cardiac glycosides[26].

B. **Borntrager's Test** (Anthraquinone Glycosides)

0.5 g of the plant extract will be shaken with benzene and organic layer got separated and half of its own volume of 10% ammonia solution will be added. A pink, red or violet coloration in the ammoniacal phase indicated the presence of anthraquinone glycosides [26].

2.2. 6. Test for Amino Acids (MILLON'S TEST)

2 ml of Millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid), were added to 2 ml of the extract. Changing the colour of the resulting precipitate from white to red upon gentle heating indicates the presence of amino acids [26].

2.2. 8. Test for Carbohydrates

A- MOLISCH'S TEST: The extract was treated with a few drops of alcoholic α-napthol. Then 0.2 ml of concentrated sulphuric acid was slowly added along the sides of the test tube. Appearance of a purple to violet colour ring at the junction indicates the presence of carbohydrates.

B- FEHLING'S TEST: Equal volume of Fehling's A (Copper sulphate in distilled water) and Fehling's B (Potassium tartrate and Sodium hydroxide in distilled water) reagents were mixed and a few drops of the sample were added and boiled, a brick red precipitate of cuprous oxide indicates the presence of reducing sugars[1].

2.2. 9. Test For Sterols and Triterpenoids (SALKOWSKI'S TEST)

The extract was treated in chloroform with a few drops of concentrated sulphuric acid, and then the mixture was shaken well and was allowed to stand for some times. Appearance of red colour in the lower layer indicates the presence of sterols and the appearance of yellow colour in the lower layer indicates the presence of triterpenoids [28]

2.3. Antimicrobial Tests

The extracts of the stem bark of *Balanites aegyptiaca* was screened for the antimicrobial (antibacterial and antifungal) tests. The tests were carried out at the Medicinal and Aromatic Plants Research Institute, National Center for Research, Khartoum, Sudan.

2.3.1. Test Microorganisms

B. aegyptiaca stem bark was screened for its antimicrobial activity against six standard bacteria, one gram-positive bacteria strain (*staphylococcus aureus*), five gram-negative bacteria strains (Escherichia coli, Salmonella typhi, Proteus vulgaris, Klebsiella pneumoniae and Pseudomonas aeruginosa) and two fungal strains (Aspergillus niger and Candida albicans) using the cup plate agar diffusion method.

2.3.2. Preparation of the Test Organisms

2.3.2.1. Preparation of Bacterial Suspension

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed on to nutrient agar slopes and incubated at 37 ºC for 24 hours. The bacterial broth was harvested and washed off with 100 ml sterial normal saline to opacity of matched barium chloride turbidity standard the suspension was stored in the refrigerator at 4 ºC till used[33].

2.3.2.2. Preparation of Fungal Suspensions

The fungal cultures were maintained on Sabouraud Dextrose Agar, incubated at 25 ºC for 4 days. The fungal growth was harvested and washed off with 100 ml sterial normal saline and the suspension was stored in the refrigerator at 4 ºC till used [33].

2.3.3. Test of Extracts for Antimicrobial Activity

2.3.3.1. Test for Antibacterial Activity

The Cup-Plate Agar diffusion method was adopted with some minor modification 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 molten sterile nutrient agar which was maintained at 45 ºC. 20 ml aliquots of this incubated nutrient agar were distributed into sterile petri-dishes. The agars was left to set and in each of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer and the agar discs were removed. Alternate cups were filled with 0.1 ml sample of each extracts using automatic microliter pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 ºC for 18 hours. After incubation the diameters of the resultant growth inhibition zones were measured [33].

2.3.3.2. Test for Antifungal Activity

The same method as for bacteria was adopted. Instead of Muller Hinton agar, Sabouraud Dextrose Agar was used. The inoculated medium was incubated at 25 ºC for two days for *Candida albicans* and three days for *Aspergillus Niger*.

RESULTS AND DISCUSSION

3.1. Phytochemical Screening

The phytochemical investigation revealed the presence of some major bioactive compounds namely: Alkaloids, Tannins, Triterpenoids, Saponins in addition to the presence of two biomolecules Amino Acids and Carbohydrates, but it didn't detected the presence of Flavonoids, Glycosides and Sterols (Table 1).

As shown in table (2), the findings of this study agreed with the findings of Dubey [18], and Wufem [16] in regard to the presence of Tannins, Triterpenoids and Saponins in the methanol extract of the *B. aegyptiaca* stem bark. Also they agreed with the results of Dahawi [17] in regard to the presence of Triterpenoids and Saponins. However, it differs from the results of all the above three authors in regard to the alkaloids which were detected by this study but not detected by them. Another author Sarker [7], who used the same methanol extract, was detected the presence of alkaloids in the plant stem bark. Several previous phytochemical studies [e.g. 13, 14, 15] were detected the presence of alkaloids in the stem bark but with other extracts rather than methanol. These controversial results between the different authors regarding the presence or absence of Alkaloids in the plant stem bark may be attributed to the variation in the environmental factors that influence the plant growth in its different habitats. On the other hand these results are in line with Wufem [16] and Dubey [18] in regards to the absence of Flavonoids, Glycosides and Sterols in the methanol extract of the *B. aegyptiaca* stem bark. However, several studies [e.g. 13, 14, 15, 17] did detect these compounds in this part of the plant but with ethanol and aqueous extracts. This may indicate that the detected compounds may vary with the variation of the solvents.

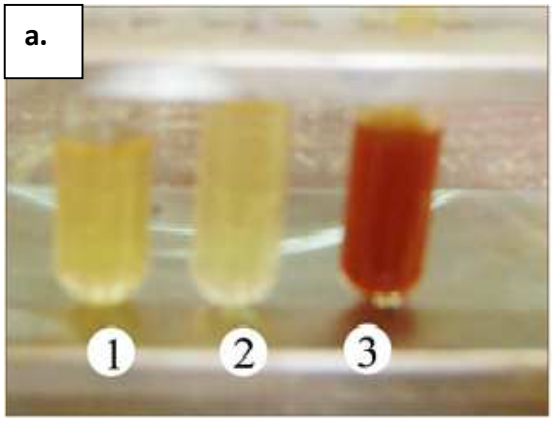
The results of this study also revealed the presence of two biomolecules Carbohydrate and Amino Acid, and this are in line with other phytochemical studies [e.g. 14, 15, 34] which proved the presence of these two compounds in the plant stem bark (*see table* 3).

It worth mentioning that, among all the phytochemical compounds detected by this study saponins is the only compound which was detected in the extracts of all the others plant organs (seeds, fruits, flowers, leaves and roots) as reported by many previous studies [19,20,35,36].

Table (1): The results of the phytochemical screening

	Phytoconstituent	Inference extract
1	Alkaloids	+
2	Flavonoids Test A , Test B and Test C	 -
3	Tannins	+
4	Saponins	+
5	Glycosides Keller killiani test (Cardiac glycosides) Borntrager's test (anthraquinone glycosides)	 - -
6	Amino acids millon's test	 +
7	Carbohydrates molisch's test fehling's test	 + +
8	Sterols salkowski's tes	 -
9	Triterpenoids salkowski's tes	 +

Key: + = Present and - = Absent



Test of Alkaloids

Test of Tannins

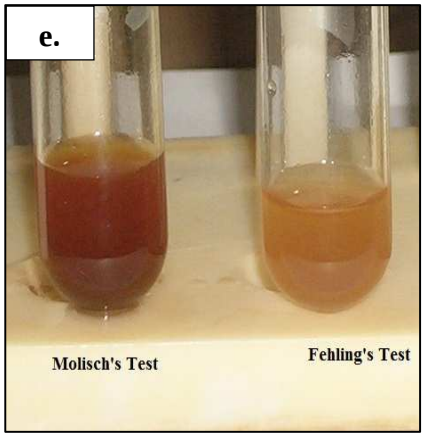
Test of Saponins

1- Sample without detecting reagent

2- Sample + Mayer reagent

3- Sample + Wagner reagent

Test of Amino Acid



Test of Carbohydrate

Test of Triterpenoids

Fig.1. Colors test of phytoconstituent compounds of *Balanite aegyptiaca* stem bark (a– f)

Table (2): Summary of the findings of this study compared to the findings of the previous phytochemical studies of *B. aegyptiaca* stem bark

Author	Extract	Al	Fl	T	Tr	Sa	Gl	A.A	Ca	St
This study	MeOH	+	-	+	+	+	-	+	+	-
7	MeOH	+	N	N	N	N	N	N	N	N
13	Aq.	+	-	+	N	+	-	N	N	N
14	EtoH	+	+	+	N	-	+	+	+	+
15	-	+	+	N	N	+	-	+	+	N
16	MeOH	-	-	+	+	+	-	N	N	N
17	EtOH	-	-	-	+	+	+	N	N	N
18	MeOH	-	-	+	+	+	-	N	N	N
37	Aq.	-	+	N	N	-	-	N	N	-

Key: Al = Alkaloids, Fl = flavonoids, T = Tannins, Tr = Triterpenoids, Sa= Saponins, Gl = Glycosides, A.A = Amino acids, Ca=Carbohydrates, St = Sterols. MeOH = Methanol extract, EtOH= Ethanol Extract, Aq= Aqueous Extract. N= Not screened

3.2. Biological Activity

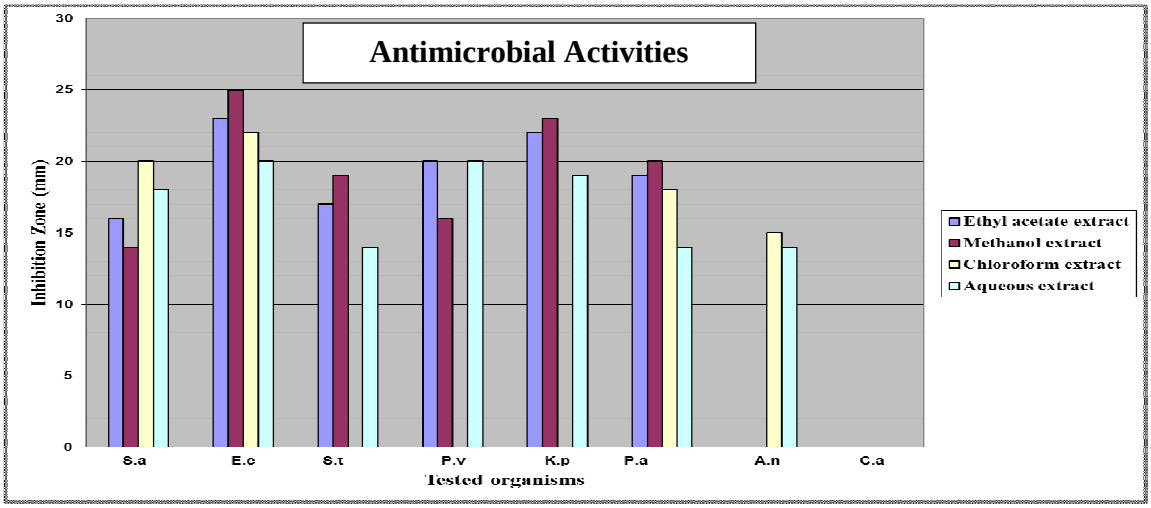
Table (3) shows that the extracts of the stem bark of the plant exhibited antibacterial and antifungal activity. All the extracts revealed antibacterial activity against all bacteria species (with inhibition zones ranging from 14 – 25 mm) except the chloroform extract which revealed antibacterial activity against only three bacterial species namely: *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* with inhibition zones 22, 20 and 18 mm respectively. Although the results indicate a clear variation in the inhibition zones of each extract against the tested bacteria as well as variation among the four different extracts (*refer to* Fig. 2), all the four extracts exhibited their highest antibacterial activity against *E-coli*, with methanol extract revealed the highest effect (25 mm) followed by ethyl acetate extract (23 mm) and then chloroform extract (22nm) and latest the aqueous extract (20 mm). All the four extracts revealed antibacterial activity against *Staphylococcus aureus* with the chloroform extract showing the highest activity (20 mm) among all followed by the aqueous extract (18 mm), whereas ethyl acetate and methanol extracts showed their lower antibacterial activity against this species (16 mm, 14 mm respectively). Fig.3. shows the highest two inhibition effects of each of the four extracts against the tested bacteria.

On the basis of these results, it is possible to state that the methanol extract exhibited the best antibacterial activity against the tested bacterial species as it showed the highest effect against four of the six tested bacteria namely, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella typhi*.

A very recent study [19, 38] reported that *Balanite aegyptiaca* has an antibacterial activity as the plant extracts inhibited the in vitro growth of several strains of staphylococcus *aureus* and *Pseudomonas aeruginosa*. Another study [39] also found that the aqueous leaves extracts of *B. aegyptiaca* have antibacterial effect against *S. typhi* isolated from blood clod culture. The presence of Saponins, Terpenoids and Alkaloids may be responsible for the antimicrobial activity of this plant as reported also by some earlier studies [22, 36, 40, 41]. Furthermore, these results were supported by Valarmathi [42] who claimed that the curative effect of *Balanite aegyptiaca* in the treatment of wounds could be linked with its antibacterial activity against s*taphylococcus aureus* and *Pseudomonas aeruginosa* which are the most common bacteria implicated in wounds.

With regard to the antifungal activity of *Balanites aegyptiaca* stem bark, our results didn't reveal any significant antifungal effect as two of the four extracts (Ethyl acetate and Methanol) didn't show any activity, whereas, the others two (Chloroform and Aqueous) demonstrated very weak antifungal activities with inhibition zones 15 , 14 mm respectively (*see* table 3 and Fig. 4). In contrast to these results, Maregesi [43] reported that the same methanolic extract of *Balanites aegyptiaca* stem bark exhibited antifungal activity against *Candida albicans* with minimum inhibition concentration (MIC) 125 g/ml. This study is not able to provide any justification for this difference. It worth mentioning that some others organs of the plant namely: the roots and the fruits were reported by some studies [44, 45] to have antifungal activity against some fungi particularly *Candida albicans*. This antimicrobial activity was attributed by this study to the presence of the above bioactive compounds mainly the Saponins, Terpenoids and Alkaloids as suggested also by [36,41].

Fig. 2. A histogram showing the antimicrobial activities of the extracts



Key: S.a = *Staphylococcus aureus* , E.c = *Escherichia coli*, S.a = *Salmonella typhi*,
P.v = *Proteus vulgaris*, K.p = *Klebsiella pneumoniae* , P.a = *Pseudomonas aeruginosa* ,
A.n = *Aspergillus niger*, C.a = *Candida albicans*

Table (3): Antibacterial and Antifungal Activity of *Balanites aegyptiaca* Stem Bark Extracts

Organisms tested	Extracts/ Zone diameter of growth inhibition (in mm)				
	No. of Type	Ethyl acetate extract	Methanol extract	Chloroform extract	Aqueous extract
Staphylococcus aureus	ATCC 25923 G+ve	16	14	20	18
Escherichia coli	ATCC 25922 G-ve	23	25	22	20
Salmonella typhi	NCTC 0650 G -ve	17	19	-	14
Proteus vulgaris	NCTC 8193 G -ve	20	16	-	20
Klebsiella pneumoniae	ATCC 53657 G -ve	22	23	-	19
Pseudomonas aeruginosa	ATCC 27853 G-ve	19	20	18	14
Aspergillus niger	ATCC 9763	-	-	15	14
Candida albicans	ATCC 7596	-	-	-	-

NCTC: is The National Collection of Type Culture, Colindale England.
ATCC: is The American Type Culture Collection, Rockville Maryland, USA.
Result: >18 mm: Sensitive, 14 to 18 mm: Intermediate: <14 mm: Resistant.

(-): No inhibition zone.

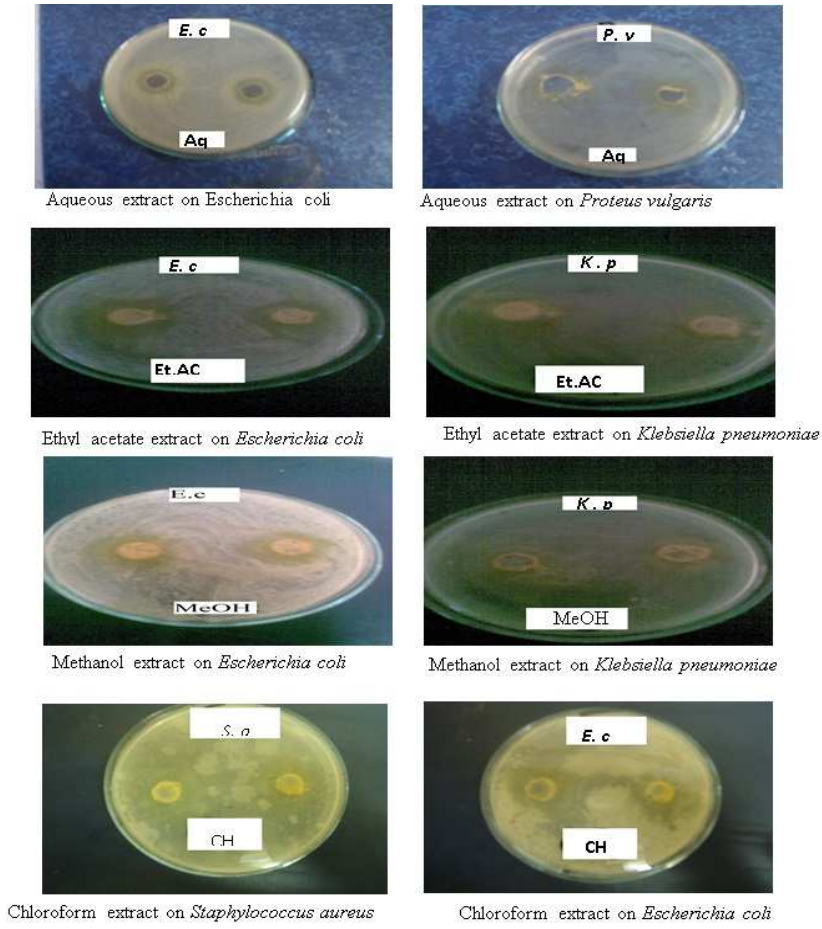


Fig. 3. The highest two inhibition effects of each of the four extracts against the tested bacteria

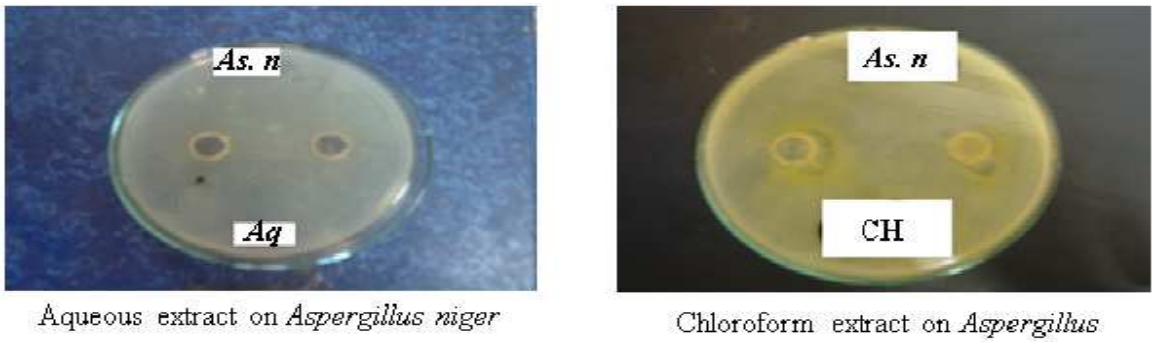


Fig. 4. The inhibition effects of the two extracts against the tested fungi

CONCLUSION

The study proved that *B. aegyptiaca* possesses several bioactive phytochemical compounds namely: Alkaloids and Saponins which are responsible for the antimicrobial and antilarval activity. The antimicrobial investigation revealed that most of the extracts have moderate to high inhibitory effects against most of the tested gram positive and gram

negative bacterial strains, while it revealed a very weak effect against the tested fungal species. These properties of the plant are in good agreement with the traditional uses in different health remedies.

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