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Phytochemical Screening and Antimicrobial Activity of Different Extractions of Sudanese *Stylochiton borumensis* Roots (Araceae)

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Abstract: *Stylochiton borumensis* distributed throughout central and Western Sudan in Kordofan, its roots are used to relief the pains of scorpion stings, the present study was conducted to investigate the chemical constituents of *Stylochiton borumensis* (Araceae) and to evaluate its potential antimicrobial activity. The roots of *Stylochiton borumensis* were collected from EL-Debabat city, South Kordofan State-Sudan. Extractions with different solvents were done, screening of extracts for antimicrobial activity, dried discs were prepared for susceptibility assays. The plates were inoculated for sensitivity determination, minimum inhibitory concentration (MIC) were determined and phytochemical screening was carried out. The findings of three (n-hexane, ethyl acetate and methanol) extracts were done through a serial of extraction method with increasing order of solvents polarity. This extracts at different concentration ranging from 100mg/ml to 6.25 mg/ml were used to test antibacterial and antifungal activities, using paper disc diffusion assay against four standard microorganisms, two gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), two gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and one antifungal (*Candida albicans*). The methanolic extract showed high activity against *Bacillus subtilis* (18mm) and moderate against *Pseudomonas aeruginosa* (15mm). The ethyl acetate extract showed inactive activity against *Pseudomonas aeruginosan* (12 mm) and the n-hexane extract against *Escherichia coli* (10 mm). Methanolic extract revealed that the *S. borumensis* roots had a significant potential effect to inhibit the growth of both bacterial and fungal strains and this may be due to the presence of alkaloids, tannins, saponins and flavonoids.

Key words: Extraction · Phytochemical Screening · Antimicrobial Activity · *Stylochiton borumensis*

INTRODUCTION

Nowadays, diseases transmitted by microbes such as fungi and other bacteria are one of the health problems in many countries worldwide. A Gram-positive bacterium like *Staphylococcus aureus* causes several serious problems such as food poisoning, postoperative endocarditis, osteomyelitis, toxic shock syndrome and wound infections [1]. People infected with antimicrobial-resistant strains are more likely to have longer, more expensive hospital stays and may be more likely to die as a result of the infections; this problem has forced researchers to search for new antimicrobial substances from various sources as novel antimicrobial therapeutic agents. One of these novel antimicrobial sources are the plants which are very rich source for a wide variety of phytochemicals such as alkaloids, terpenoids, saponins, flavonoids, phenols and tannins [2], which have been reported to have biological activities. Medicinal plants and derived products are used for the treatment of major disease such as typhoid fever, cardiac edema, diabetes, malaria, obesity and high blood pressure [3]. Likewise, herbal drugs have been in use by different civilizations in different parts of the world for centuries to fight a large number of diseases.

Stylochiton borumensis (Araceae) is one of the 150 species available from the genus Stylochiton Scott. Locally names in Sudan are Moura or Arrag Alagrab. (Figure 1) *S. borumensis* is erect annual herbs with thick fleshy rhizomes. Leaves radical, petiolate; laminas

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Fig. 1: Morphology appearance of *S. borumensis* in its natural habitats

sagittate, 7-11 X 3-5cm, apex cuspidate or obtuse base attenuate, sheathing at the base up to 7 cm long with undulating margins. Flowers monoecious, stamens monadelphous [4]. *S. borumensis* was reported in world widespread in subtropical and tropical regions, throughout central and Western Sudan in Kordofan and [5]. The roots are used in Sudanese traditional medicine to relief the pains of scorpion stings [5]. Phytochemical investigations of *S. borumensis*, leaves revealed the presence of contain flavonoids, tannins, alkaloids, glycosides, total polyphenols, flavonoids and total tannins, in addition to essential and non -essential amino acids [6]. Previous studies on the leaves of *S. borumensis* confirmed that the petroleum ether and chloroform extracts were not active against antibacterial but the extracts showed high antifungal activity, cytotoxcity against Brine Shrimps lethality and antioxidant activities [6].

This study aimed to assess the antimicrobial activities of *Stylochiton borumensis* roots with determination of chemical constituents of this roots.

MATERIALS AND METHODS

Collection and Identification of the Plant: Root of the selected plant was collected from Elfaki Musa village in EL-Debabat city, South Kordofan State-Sudan, with the help of the informants, September 2015 according to Issa 2018 [7]. Only plants judged to be mature and disease-free were harvested in morning hours. The plant was identified by Medicinal and Aromatic Plants Research Institute at Herbarium Department. The plant specimen was deposited at Herbarium under Voucher Number U.B.H.5/9/2015 and kept in Bahri University Herbarium in the Department of Botany.

Preparation of Plant Material for Extraction Experiments: This was done under laboratory conditions at college of Applied and Industrial Sciences University of Bahri, Alkadro area. Bahri state, Northern Khartoum, Sudan.

The plant material was sorted and cleaned to improve on quality and the roots were air-dried to constant weight in the shade at room temperature. The dried roots were then crushed into powder using hammer and mill, sieved and packaged into clean cloth bags until when needed.

Extraction: A sample of (100g) of the dried ground powdered roots of *S. borumensis* were extracted successively by cold method using Shaker apparatus; with n-hexane, ethyl acetate and finally extracted with methanol [8, 9].

Concentration of Each Crude Extracts from Extracting Solvents: Each extract was filtered and allowed to evaporate to a constant weight under vacuum room temperature at 37°C, to avoid high temperatures cold method were applied to reduced inactivation of active ingredients. The yields and a percentage yield of extracts were tabulated in Table 1. The crude extract was then stored at 4°C awaiting screening. This experiment was designed for the purpose of assessing the effect of *S. borumensis* roots three extracts in comparison with references drugs [9, 10].

Preparation of Standard Microorganism's Suspensions: Preserved bacterial strains were cultured on nutrient broth, prior to antibacterial susceptibility testing. Full loop of standard bacteria was inoculated in nutrient broth at 37°C in incubator for 18-24 hours. The bacterial growth was observed, the broth was sub-culture to nutrient agar

Table 1: Yield and physical appearance of the different extracts of *Stylochiton borumensis* roots

	Successive extraction with		
Properties	n-hexane	ethyl acetate	methanol
Characteristic	Semi-oily	Semi oil (wax)	Greasy gummy
Color	Yellow Brown	Creamish	Yellow
Yields (gram)	0.48	3.3	2.03
Yield (%)	0.24%	1.65%	1.01%

plates for 24 hours. When growth evidenced, the individual colonies were again sub-cultured to nutrient agar slopes and inoculated at 37°C in incubator until the growth occurred. 1 ml of the bacteria suspension was diluted with 9 ml of sterile saline (1:10) that was taken with digital pipette and shaken gently to produce a suspension was adjusted (a turbidity corresponding to 0.5 McFarland standards No.1) that containing about (10^8-10^9CFU/ml) colony-forming unit per ml. The suspension was stored in the refrigerator at 4°C till used. Preserved fungal strains were cultured on Saboraud dextrose agar, prior to antifungal susceptibility testing. The supernatant was adjusted to a turbidity of opacity equivalent to Mc Farland standard No.1. Then 1 ml of the suspension was diluted with 4 ml of sterile saline and used to inoculate each of the drug-containing media as well as the controls [8].

Test microorganisms

Name of standard organism	Type of organism	ATCC code
Escherichia coli (*E. c*)	Gram negative bacteria	25922
Pseudomonas aeruginosa (*P. a*)	gram negative bacteria	27853
Bacillus subtilis (*B. s*)	gram positive bacteria	8236
Staphylococcus aureus (*S. a*)	gram positive bacteria	25923
Candida albicans (*C. a*)	Fungi	7596

Note: ATCC = American Type Culture Collection

Screening of Extracts for Antimicrobial Activity: Antimicrobial activity was carried out using paper disc diffusion method described by Bauer *et al*. [9]. To screen the antibacterial and antifungal activity of plant extracts were performed by using Mueller Hinton agar (MHA) medium for bacterial organisms and Sabouraud dextrose agar (SDA) medium for fungi organism. The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines [10].

Preparation of Dried Discs for Susceptibility Assays: Whatman filter paper (No.1, 6 mm in diameter) was used to make bio-discs (6 mm in diameter). The discs were sterilized by autoclaving.

Preparation of Plant Extracts Concentrations for Antimicrobial Assays: Then various concentrations (100, 50, 25, 12.50 and 6.12 mg/ml) of plant extract were prepared in Dimethylsulphoxide (DMSO).

Preparation of Standard Drug Concentrations for Antimicrobial Assays: Bacterial standard drugs Ampicillin 20µg/ml, Gintamicin 10µg/ml and Ciprofloxacin 5µg/ml and fungal standard drugs Griseofulyin 5µg/ml and 25µg/ml and Nystatin 5µg/ml and 25µg/ml were prepared. The discs were then placed onto sterile Petri dishes and then followed the same procedure as for bacterial and fungal assays. One mg of the standard drug per disc.

Inoculation of Plates for Sensitivity Determination: Each of bacterial and fungal suspensions were diluted with sterile solution to 10^8cfu/ ml (turbidity corresponding to 0.5 McFarland standards). One hundred microliters (1 ml) of the inoculum of bacterial and fungal suspensions were pipetted onto each of the MHA and SDA plates respectively and distributed uniformly by using swabbed over the surface of the medium by gently rocking the plate and excess fluid was pipetted off. Then, the plates were placed in an incubator for 10 min to dry before the discs were applied.

Application of Discs and Incubation of Petri Dishes: Within 15 min after the inoculation of plates, the sterilized discs found in sterile Petri dishes, were placed with flamed forceps on the surface of the MHA of bacterial strain cultured one of each quadrant of the Petri dish, pressed down with slight pressure in order to ensure complete contact with the MHA agar surface and dispensed with 20 µl of a solution of each plant extracts in the middle of each disc, giving a minimum of 6 and 1 mg of extract per disc. They were then left to dry for 1 h. Then the plates were incubated at 37°C for 24 h in the inverted position. The same manner were applied for and references drugs, but fungal strains by using SDA agar and left for aerobic incubation for 48 and 72 h in an inverted position for *C. albicans* and other dermatophytes, respectively. The diameters of the inhibition zones in each case were then measured in millimeters scale (mm). The experiments were done in twice and the mean of the results determined (Figure 2 and Figure 3).

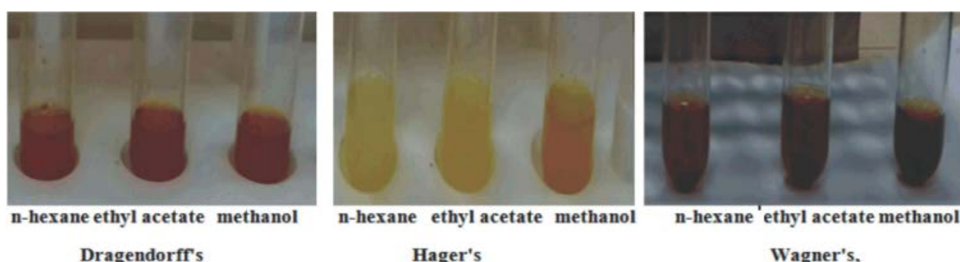


Fig. 2: Tests of Dragendorff's, Hager's and Wagner's for detection of Alkaloids

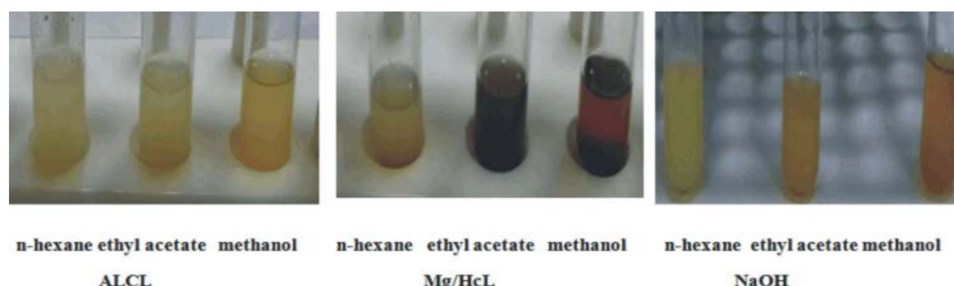


Fig. 3: Test of $ALCL_3$, Mg/ H_2SO_4 and NaOH for detection of Flavonoids

Determination of the Minimum Inhibitory Concentration (MIC): MIC values were determined as followed guidelines by Prescott, Harley and Kelein [11] and Manna, Banso and Clifford [12] who stated that screening plant extracts against the test organisms normally shows that bacteria and fungi vary widely in the degree of their susceptibility to antibacterial and antifungal agents respectively. Determination of minimum bactericidal and fungicidal concentrations was based on findings [13] who suggested that some plant extracts have antibacterial and antifungal agents that are bacteristatic and fungistatic at lower concentrations while becoming bactericidal and fungicidal at higher concentrations.

Phytochemical Screening: General photochemical screening for the active constituents such as alkaloids, flavonoids, tannins and saponins etc., on the extracts with few modifications was carried out by using the standard methods described by Farnsworth *et al.* Harbone and Sofowora [14-16].

Test for Akaloids: Three ml of extract was poured on petri dish and dried in water path, then dissolved in ten ml of HCL 2% or NH_4OH 10% and transferred in three test-tubes each one contain one ml, few drops of the following reagent were added into each tube (Dragedorff's gave orange precipitation, Wagner's gave reddish precipitation and Hager's gave yellow precipitation which indicated the present of alkaloids.

Test for Flavonoids: Two ml of extract evaporated on petri dishes and then ten ml of ethanol were added, then transferred into four test tubes, the one added 1ml of 1% NaOH that give yellow color, the second test tube was poured a few powder of magnesium turnate piece followed by adding concentrated HCL, the formation of a pink, crimson red which indicate the present of Flavonoid, the third test tube was treated with 1 ml of 10% $ALCL_3$ solution, the formation of creamy color indicated the present of Flavonoid, the fourth test tube was treated with Ammonium solution, the formation of yellow/orange color indicated the present of flavonoid.

Test for Saponins: Two ml of extracts was concentrated in water bath, then was shaken with 5 ml of distilled water in a test tube. Frothing which persists on warming was taken as evidence for the presence of saponins (Figure 4).

Test for Tannins: Two ml of extracts was stirred with 1 ml of distilled water, filtered and few drops of ferric chloride were added to the filtrate. A blue-black, green or blue-green precipitate was taken as evidence for the presence of tannins (Figure 5).

Test for Triterpenes and Sterols: Two ml of extract was dried in water path and dissolved in 6ml of chloroform, a few drops of concentrated sulfuric acid were added along the side of the test tube two layers was formed, the upper

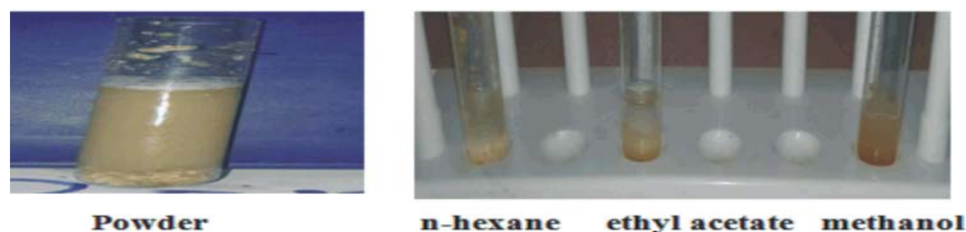


Fig. 4: Test of Foam for detection of Saponins

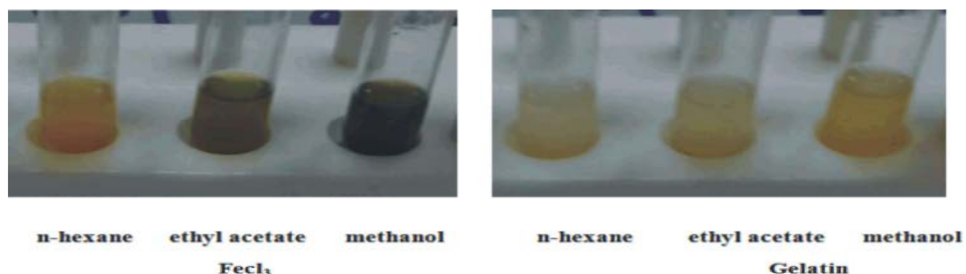


Fig. 5: Test of ferric cholride and gelatin for detection of tannins

green color indicated the presence of sterol and the middle red brown ring indicated the presence of triterpenes.

Test of Cyanogenic Glycosides: 2g of the powdered plant sample was placed in erlen Mayer flask and sufficient water (50ml) was added to moisten the sample, flowed by 1ml of chloroform (to enhance enzymes activity). Apiece of freshly prepared sodium (yellow) Bal jet's reagent was carefully inserted between split crocks which was used to stopper the flask, A change in color of the sodium picrate paper from yellow to various shades of red (orange) was taken as an indication of the presence of Cyanogenic Glycoside.

RESULTS

The yield of the three successive solvents extracts (n-hexane, ethyl acetate and methanol) and physical appearance are depicted in Table 1.

The results of the general phytochemical screening for the active constituents indicated the presence of many phytochemical analyses such as alkaloids, flavonoids, tannins and saponins etc., in the ethanolic and other three sequential extractions such as (n-hexane, ethyl acetate and methanolic extracts) of *Stylochiton borumensis* roots given in Table 2 and Figures (2, 3, 4 and 5)

The mean diameters of inhibition zone (MDIZ) produced by candidate extracts on standard microorganisms are presented in Figure 6 and Figure 7.

Table 2: Phytochemical screening of *Stylochiton borumensis* roots

		Successive extraction with		Single	
Secondary metabolites	Tests	n-hexane	ethyl acetate	Methanol	Ethanol 80%
Alkaloids	Drogndroff's	+++	++++	++++	++++
	Hager's	+++	+++	+++	++++
	Wagner's	+++	++++	++++	++++
Flavonoids	1%Na OH	++	++	++++	+++
	NH_4OH	++	+++	++++	+++
	10%$ALCL_3$	++++	++++	++++	++++
	Mg/HcL	-	++	+++	+++
Saponins	Foam	-	-	+	+
Tannins	$FeCl_3$	-	+	++++	++++
	10% Gelatin salt	-	+	++	++
Sterols/Triterpene	Liebermman's	+++/+	++/+	++/++	++/++
	Salkowski	+++/+	++/+	++/+	++/++
Coumarin	KOH/UV	+++	+++	+++	+++
Glycosides	Cyanogenic	-	-	-	+

++++ = very high concentration; +++ = high concentration; ++ = moderate concentration; + = trace amount and - = absent.

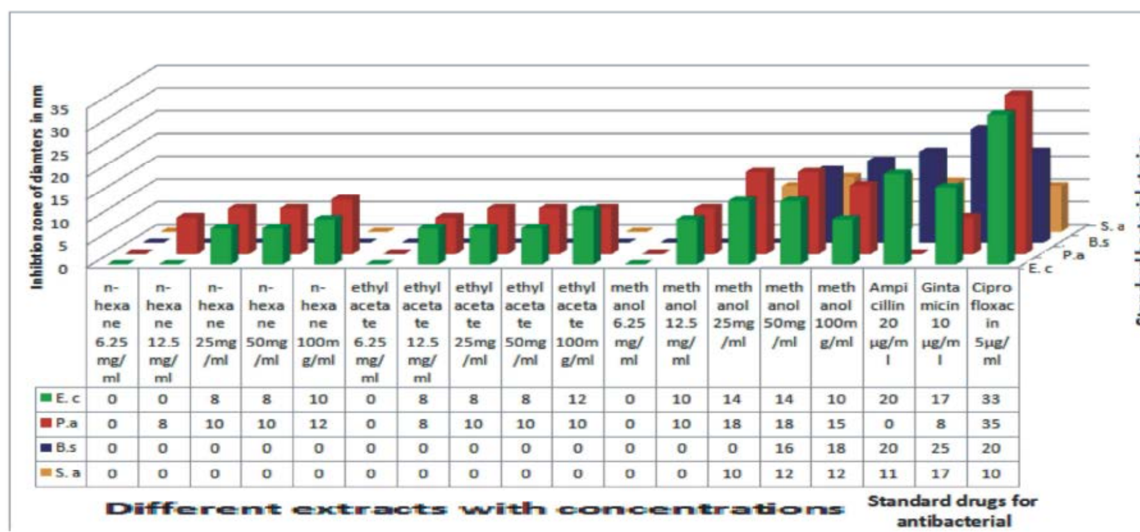


Fig. 6: Antibacterial activity of roots different extracts at concentrations 6.25, 12.50, 25, 50 and 100 mg ml^{-1} of *Stylochiton borumensis* against standard bacterial strains and compared with references standard drugs

Key: Standard microorganisms (*E.c =Escherichia coli; P.a =Pseudomonas aeruginosa*; *B.s =Bacillus subtilis*; *S.a = Staphylococcus aureus*) Notice: the values were determined in duplicate by Mean diameter inhibition zone (MDIZ) in mm

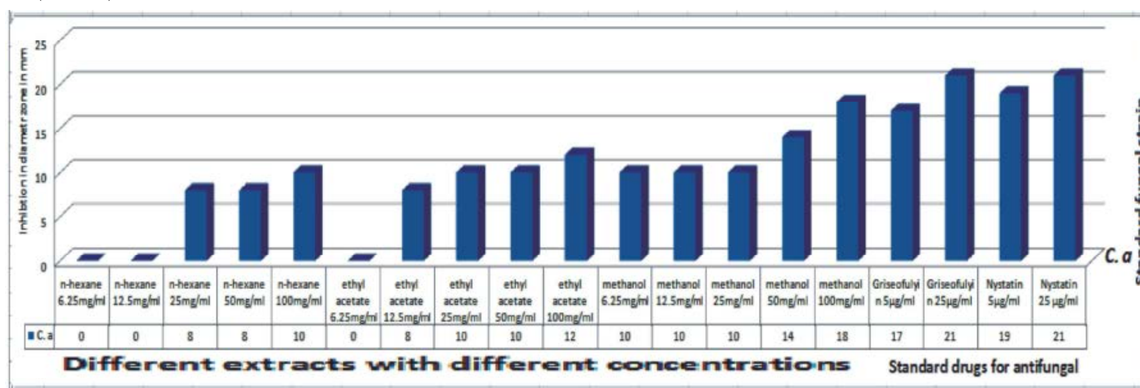


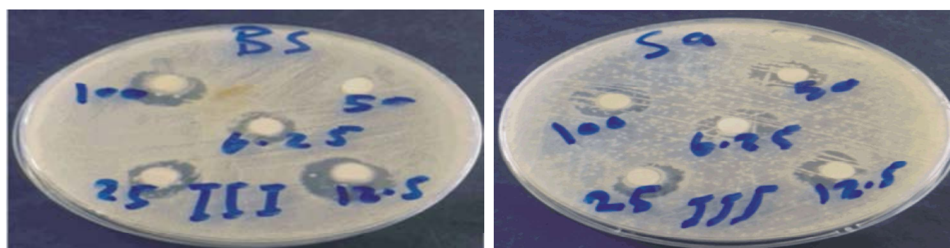
Fig. 7: Antifungal activity of roots different extracts at concentrations 6.25, 12.50, 25, 50 and 100 mg ml^{-1} of *Stylochiton borumensis* against standard fungal strain and compared with references standard drugs

Key: Standard microorganisms (*C.a =Candida albicans*), Notice: the values were determined in duplicate by Mean diameter inhibition zone (MDIZ) in mm.

On the other hand, Figures (6 and 7) showed antimicrobial activity of the reference chemotherapeutic drugs against the test microorganisms. The results were interpreted as sensitive, intermediate and resistant. Based on the results of the plant extracts resulting <9mm zone was considered as inactive; 9-12mm as partially active; while13-18mm as active and >18mm as very active [17-24].

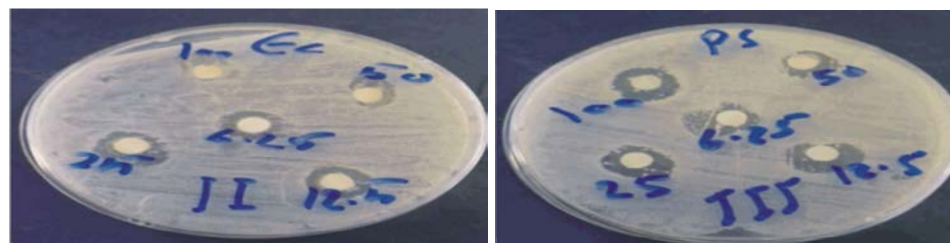
DISCUSSION

The antibacterial activity of different extracts of *Stylochiton borumensis* roots. The result of methanol extract at 100 mg/ml gave high activity against *E. coli, P. aeruginosa* plate (2 number D) and *S. aureus* plate (1 number B) with inhibition zone (18, 18 and 18 mm) respectively more than reference Gentamicin (10µg/disk) for *E. coli* gave a zone of 17 mm and *P. aeruginosa* 8 mm. Different roots extracts showed antibacterial activity against both gram negative *E. coli* and *P. aeruginosa* all extracts gave activity. The methanol extract were susceptible against *B. subtilis* plate (1 number A) with inhibition zones ranged from (12) mm. The methanol extract showed the highest activity (18 mm) followed by the ethyl acetate (12 mm) and n-hexane (12 mm) extracts



A = *Bacillus subtilis* **B=** *Staphylococcus aureus*

Plate 1: Antibacterial activity of *S.borumensis* roots against two gram positive bacterial organisms with different concentrations of methanol extract



C = *Escherichia coli* **D=** *Pseudomonas aeruginosa*

Plate 2: Antibacterial activity of *S.borumensis* roots against two gram negative bacterial organisms with different concentrations of ethyl acetate and methanol extract respectively

Key word II= using ethyl acetate extract. III= using methanol extract

E.c =Escherichia coli; *P.a =Pseudomonas aeruginosa*; *B.s =Bacillus subtilis*; *S.a = Staphylococcus aureus*

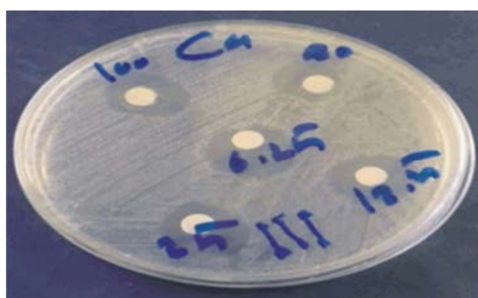


Plate 2: Antifungal activity of *S.borumensis* roots against *Candida albicans* organisms with different concentrations of methanol extract

Key word III= using methanol extract

Minimum Inhibitory Concentrations of *Stylochiton borumensis*

respectively but the ethyl acetate and n-hexane extract was inactive against *B. subtilis*. The methanol showed potent antibacterial activity against *S. aureus* plate (1 number B) with inhibition zone of 18 mm. Generally, the methanol plates (1 number A & B and 2 number D) and ethyl acetate extracts plate (2 number C) showed higher antibacterial activity than the n-hexane extract. The n-hexane of roots extract was inactive against *Staphylococcus aureus*, *Bacillus subtilis* and *P. aeruginosa.* The ethyl acetate extract was inactive against *Staphylococcus aureus* and *Bacillus subtilis*. The ethyl acetate, n-hexane and methanol extracts of *S. borumensis* showed activity ranged from (12-18 mm) higher than that obtained for Gintamicin at 10 mcg/disc (8 mm) but lower than that displayed by ciprofloxacin at 5 mcg/disc (35 mm). As showed in Figure 6.

All roots extracts exhibited antifungal activity against *C. albicans* with inhibition zone value of 18 mm for the methanol extract Plate 2 higher than that obtained for Griseofulyin but lower than that displayed by Nystatin, 12 mm for the ethyl acetate extract and n-hexane with inhibition zone 10 mm this lower than that displayed Nystatin and Griseofulyin. As showed in Figure 7 and Plate 2.

The minimum inhibitory concentrations (MICs) of most active extracts methanol, ethyl acetate and n- hexane of *S. borumensis* roots against standard organism were given in (Table 3). MIC of the different extracts was tested at different concentration by serial dilution to give concentration (100, 50, 25, 12.5 and 6.25µg /ml). The methanolic extract of *S. borumensis* roots gave the highest minimum inhibitory concentration against *E. coli* (MIC value 12.5mg/ml), *P. aeruginosa*

Table 3: Minimum Inhibitory Concentrations of *Stylochiton borumensis* roots against Standard Microorganisms

	MIC of standard microorganisms mg/ml				
Extracts	*E. c*	*P.a*	*B.s*	*S.a*	*C.a*
n-hexane	12.5	6.25	ND	ND	12.5
Ethyl acetate	12.5	12.5	ND	ND	12.5
Methanol	12.5	12.5	50	25	6.25

Key: ND= not determined

(MIC value 12.5mg/ml), *S. aureus* (MIC value 25 mg/ml) and *C. albicans* (MIC value 6.25 mg/ml). The ethyl acetate and n-hexane extracts were found to be inactive against *S. aureus* and *B. subitilis*.

CONCLUSION

Antimicrobial resistance is reported to be on the increase due to gene mutations of the disease causing pathogens. It is believed that, new antibiotics with activities and structures different from those in current use could be found through ethno botanical route. *Stylochiton borumensis* was chosen for this study because of their reputation in folk medicine as antimicrobial agents and usage of different part in many diseases. Phytochemical screening was carried out and lead to presence of some secondary metabolites, the plant part was showed to contain alkaloids, flavonoids, tannins, triterpenes, coumarins. The crude extracts were subjected to antibacterial assays using the disc diffusion method and the inhibition zones were measured in mm. All extracts of Roots was gave good results ranged between strong to moderate activities against all bacterial and fungal organisms were used. Methanol extract was showed as stronger growth inhibition against all organisms except *Bacillus subtilis* was showed as moderate growth inhibition against methanol extract. The n-hexane, ethyl acetate extracts was ranged not inhibition against *staphylococcus aureus* and *Bacillus subtilis* and moderate activity against others organisms.

Recommendation: The medicinal plants are continued source of new active compounds that are used for medicinal care or in drug synthesis, which must be to given more attention and support of scientific research in this area.

More research needed on this plant *Stylochiton borumensis* to specify the active component that makes inhibition for growth of microorganisms.

Further work of purification of the plant extracts to isolate the bioactive metabolites and their structure must be elucidated.

Overall, *Stylochiton borumensis* will continuous to play important role in medicine, research involving *Stylochiton borumensis* and its uses could be expanded greatly in future, leading to new treatment and cure of microbial disease.

List of Abbreviations:
MIC: Minimum inhibitory concentration
U.B.H.5/9/2015: University of Bahri Herbarium dated 5/9/2015
S. borumensis : *Stylochiton borumensis*
CFU/ml: colony-forming unit per ml
ATCC: American Type Culture Collection

E. c coli: *Escherichia coli*
P. aeruginosa: *Pseudomonas aeruginosa*
B. subtilis: *Bacillus subtilis*
S. aureus: *Staphylococcus aureus*
C. albicans: *Candida albicans*

MHA: Mueller Hinton agar
SDA: Sabouraud dextrose agar
DMSO: Dimethylsulphoxide
MDIZ: Mean diameters of inhibition zone

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