

Properties of *Stylochiton Borumensis*

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ABSTRACT

The main objective of the present paper is to determine the total phenolic content, amino acid content, carbohydrate content and heavy metal determination by Folin-Ciocalteu reagent (F-C) method, Ninhydrine method, anthrone method, inductively coupled plasma- atomic emission, respectively. And to verify the antimicrobial activity of the plant by disc diffusion method. The total phenolic content ranged from 340 to 500 mg/l gallic acid equivalent. The amino acids content the highest percentage was found in the ethanol extract (60.76%). Total carbohydrates were determined and the highest percentage was found in the ethanol extract (65.76%). Several metals including Pb, Cu, Cd and Zn were analyzed in *S. borumensis* roots by ICP-ES spectrophotometer and showed that it was in normal range compared to certified reference material the crude protein of *S. borumensis* roots was (7.15%). The ash content was a measure of the presence of inorganic compounds in drug. Ash content was (6.4%). The fiber content was (13.5%). The fats content was (2.1%). The antibacterial and antifungal activity of plant extracts with different concentrations (100, 50, 25, 12.5, 6.25mg/ml) were investigated by disk diffusion assay, four extracts were tested against five micro-organisms; two strains of Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*), two strains of Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and one fungus (*Candida albicans*). The result showed that for all micro-organisms the activity decreased by decreasing concentration of the extracts. The chloroform and ethanol 80% roots extracts gave the highest activity with inhibition zone (20mm and 17mm) respectively against *Escherichia coli* where reference antibiotic ciprofloxacin and erythromycin gave zone of (17mm and 12mm) respectively.

Keywords: Total phenolic content; antimicrobial activity; *Stylochiton borumensis* roots.

INTRODUCTION

Medicinal plant is the plant which one or more of its parts contain substances that can be used for therapeutic purpose, or a precursor for synthesis of useful drug. According to World Health Organization; phytochemistry is defined as herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes [1]. *Stylochiton borumensis* is belonging to family Araceae it is an important medicinal plant of Sudan and some other countries. The root and bark are used against convulsions, gonorrhoea, bilharzias, heart burn, stomach – ache, constipation and wound and snake bites. The ash from the burn roots mixed with porridge provides a remedy for stomach pains [2]. In Sudan the root is used to relief the pain of scorpion sting, so that it takes its local name Irig alagrab or Irig moura.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Roots of *S. borumensis* were collected by hand

from El Debatat area, South Korodfan State, Sudan, in the month of September. The plant species was identified by plant herbarium at Traditional and Aromatic plant Research Institute, National Centre for Research, Khartoum; After authentication, the plant material was dried under shade and after optimum drying, coarsely powdered and passed from sieve 40 and stored in air tight, well closed container till further use.



Fig1. *Stylochiton borumensis* roots

Estimation of Total Phenols Content (Folin-Ciocalteu Assay)

The total phenolic content was determined by

Properties of Stylochiton Borumensis

Folin-Ciocalteu reagent (F-C) method as described by Ainsworth et.al, [3]. 0.1 M of sample was mixed with 2 ml freshly prepared sodium carbonate (2%) and vortexed vigorously. After 5 minutes, 100 ml of F-C reagent (1 N) were added to the mixture, and incubated for 30 minutes at room temperature. A blue color was developed in each tube and intensity of the color is directly proportional to the phenolic contents. And the absorption spectra were measured in double beam spectrophotometer against blank at 750 nm calibration curve using Gallic acid. The amount of total phenolic contents were calculated as mg of Gallic acid equivalents (mg/1) using the following equation based on calibration curve;

$Y=0.0007X+0.1078$ and $R^2=0.9997$ where x equal concentration of Gallic acid (mg/1) corresponding to optical density. A calibration curve was prepared using Gallic acid (100-900 mg/1) as standard. Total phenolic content in the plant extract was calculated using formula: Total phenolic content= GAE x V/m, where GAE is gallic acid equivalence (mg/1) or concentration of gallic acid established from the calibration curve ; V is the volume of extracts in ml and m is weight (g) of the pure plant extract.

METHOD OF EXTRACTION

The coarsely powdered roots of *S.borumensis* were extracted successively with different polarity solvents like petroleum ether (40- 60 °C), chloroform, ethanol 80% and distilled water. The herb to solvent ratio was kept 1:10 to ensure complete extraction.

The plant material was extracted by cold maceration for 4, 12, 36 and 72 hours for petroleum ether, chloroform, ethanol 80% and distilled water, respectively. The extracts were filtered through Whatman filter paper to remove any precipitate, and then allowed to air dry in dishes until to dryness, after drying yield percentage of each extracts was calculated as follows: Yield%=weight of extract/weight of plant materialx100. The extracts were collected and stored at 4 °C.

Table2. Determination of heavy metals by ICP-ES for *S.bo- rumensis* roots

Heavy metal	Znmg/L	Pb mg/L	Cdmg/L	CuMg/L
Mean (Roots)	0.076	0.168	0.055	0.102
Certified value CRM1	260	73	NV	120
Certified value CRM2	13.1	0.11	NV	2.5

Before use of plant for medicinal purpose one should know the level of heavy metals in that particular plant, because if the level of a particular heavy metal is exceeding its normal

RESULTS AND DISCUSSION

The total phenolic contents ranged from 340 to 500 mg/1 gallic acid equivalent in roots. The highest content was found in the ethanol extract (500 mg/1) followed by the chloroform (440 mg/1), petroleum ether extracts (420 mg/1) and distilled water extracts (340 mg/1) respectively. Flavonoids and phenols are considered as anti-capillary fragility and anticancer compound [4].

Polyphenol have inhibitory effect on mutagenesis and carcinogenesis in human when ingested in daily diet [5].

The composition of Amino acids was performed initially with Ninhydrine chemical reagents in roots extracts of *Stylochiton borumensis*. The highest percentage was found in the ethanol extract (60.76%) followed by distilled water extracts (30.25%) respectively. Carbohydrates composition was performed initially with anthrone method in roots extracts of *Stylochiton borumensis*. The highest percentage was found in the ethanol extract (65.76%) followed by distilled water extract (40.85%) respectively.

Table1. Mean percentage of a proximate analysis of *Stylochiton borumensis* roots

No.	Parameter	roots
1	Crude protein	7.15
2	Crude fiber	13.5
3	Ash	6.4
4	Ether extract	2.1
5	Moisture	5.5
6	Dry matter	94.5
7	Nitrogen free extract	65.35

DETERMINATION OF HEAVY METALS LEVEL OF S. BORUMENSIS BY ICP- ES

Several metals including **Pb, Cu, Cd and Zn** were analyzed in *S. borumensis* roots by ICP-ES spectrophotometer. This interaction would include antagonism and synergism. Absorption of heavy metals in medicinal plants is governed by soil characteristics such as pH, salinity, conductivity and organic matter content [6].

permissible limit, it may result in serious harms to the human health as reported by [6]. Even though World Health Organization has formulated guidelines for quality assurance and control of

Properties of Stylochiton Borumensis

herbal medicine Lead well known for its adverse effects on many parts of the body Progressive exposure to lead results in a decrease in the performance of the nervous system and affects renal clearance [7]. All results above indicates that *S. borumensis* roots are within the range heavy metals when compared within certified reference material this represented in (Table 2) [7]

ANTIMICROBIAL ACTIVITY OF *S. BORUMENSIS* ROOTS EXTRACTS

African medicinal plants have been screened for their in vitro antibacterial activities and many described antibacterial activities have been focused on phenolic compounds, terpenoids and

essential oils [8] The chloroform and ethanol 80% roots extracts gave the highest activity with inhibition zone (20mm and 17mm) respectively against *Escherichia coli* where reference antibiotic ciprofloxacin and erythromycin gave zone of (17mm and 12mm) respectively.

The chloroform roots extract gave the highest anti- bacterial activity with inhibition zone 15 mm against *Pseudomonas aeruginosa*, 20 mm against *Escherichia coli*, 18 mm against *Bacillus subtilis* and 15 mm against *Staphylococcus aureus*. All root extracts showed antibacterial activity against *Escherichia coli*, all extracts gave activity with inhibition zone range from (20 to 12) mm. All the results are presented in (table 3). All extracts exhibited antifungal activity against

Table3. Antimicrobial activity of *Stylochiton borumensis* roots

Part of plant	solvent	Conc. mg/ml	Diameter of growth inhibition zone (mm)				
			bacteria				fungus
			P.s	E.coli	B.s	S.a	Ca
roots	P.E	100	11	12	11	9	9
		50	11	12	11	9	9
		25	10	11	10	8	8
		12.5	9	10	8	7	8
		6.25	8	8	7	1	7
	CHCl ₃	100	15	20	18	15	9
		50	12	10	15	14	9
		25	10	9	13	13	8
		12.5	9	8	12	12	7
		6.25	9	7	11	12	7
	EtOH80%	100	15	16	13	15	16
		50	11	12	12	14	10
		25	10	11	10	13	8
		12.5	9	10	9	12	7
		6.25	8	9	8	11	7
	D. W	100	11	17	8	11	13
		50	11	16	8	10	9
		25	10	15	7	9	8
		12.5	8	14	-	8	7
		6.25	7	13	-	7	-

Note: P.E=petroleum ether, D. W=distilled water, EtOH80% =Ethanol 80% P.s= *Pseudomonas aeruginosa*, B.s=*Bacillus subtilis*, S.a=*Staphylococcus aureus*, Ca=*Candida albicans*, E.coli=*Escherichia coli*, (-)=not active

The results were expressed in terms of the diameter of inhibition zone: < 9 mm, inactive; 9-12 mm partially active; 13-18 mm active; >18 mm very active.

Candida albicans with high inhibition zone value of (16 mm) for ethanol 80% extract, (13 mm) for distilled water extract, (9 mm) for petroleum ether and chloroform. The results are represented in table (below). Where reference antibiotics Ketoconazole and Itraconazole (40ug/ml) against *Candida albicans* gave inhibition zone (17 mm) and (16 mm), respectively. The results are

represented in (Table 3).



Fig2. Antibacterial activity of *S. borumensis* chloroform (A) and petroleum ether roots extract (100 mg/ml) (B) against *Bacillus subtilis*

Table4. Antimicrobial activity of reference antibiotics

Antibiotic	Conc.	Diameter of growth inhibition zone (mm)				
		bacteria				fungus
		E.coli	P.s	S.a	B.s	Ca
Ciprofloxacin	40 ug/ml	17	25	20	23	NT
	20	13	20	17	22	NT
	10	13	18	16	20	NT
	5	12	15	15	18	NT
Erythromycin	40 ug/ml	12	15	20	12	NT
	20	10	13	19	11	NT
	10	8	12	17	10	NT
	5	7	10	14	8	NT
Ketoconazole	40ug/ml	NT	NT	NT	NT	17
	20	NT	NT	NT	NT	16
	10	NT	NT	NT	NT	15
	5	NT	NT	NT	NT	14
Itraconazole	40ug/ml	NT	NT	NT	NT	16
	20	NT	NT	NT	NT	15
	10	NT	NT	NT	NT	14
	5	NT	NT	NT	NT	12

CONCLUSION

Stylochiton borumensis roots contain considered amount of phenolic compound which may be responsible for valuable pharmacological activities.

Phenolic compound are responsible for antioxidant, anticancer and anti-capillary fragility.

The presence of these compounds in the *S.borumensis* roots shows the medicinal importance of the plant. Further the plant could be considered for antioxidant, anti-cancer and immune modulatory activity.

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Properties of Stylochiton Borumensis

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