ISSN: 2639-7455

Research Article

Earth & Environmental Science Research & Reviews

Phytochemical Screening of Leaves and Roots of *Stylochiton Borumensis*: A Medicinal Plant

Hatim M Y[1]* and Makhawi M A[2]

[1]*Department of Biochemistry, College of Applied Sciences, University of Bahri, Sudan*

[2]*Department of Biochemistry, College of Applied Sciences, University of Bahri, Sudan*

***Corresponding author**

Hatim M.Y, Department of Biochemistry, College of Applied Sciences, University of Bahri, Sudan. E-mail: hamadnalla2009@yahoo.com

Submitted: 11 Sep 2018; **Accepted**: 15 Oct 2018; **Published**: 23 Jan 2019

Abstract

The aim of this study was to find out phytochemical classes present in various extracts of Stylochiton borumensis and to determine the total phenolic, flavonoids and tannins content in different plant extracts. Total phenols, flavonoids and tannins content was determined by folin-ciocalteu assay, aluminum chloride colorimetric assay and ferric chloride colorimetric assay respectively. Different extracts of S. Borumensis showed the presence of alkaloids, sterols/ triterpenoid, flavonoids, tannins and coumarins. The phenolic, flavonoids and tannins contents of plant extracts using gallic acid (Y= 0.0007x + 0.1078, r^2 = 0.9997), quercetin (Y= 0.0007x + 0.053, r^2= 0.9984) and tannic acid (Y= 0.0009x + 0.043) as standards. The total phenolic content ranged from 460 to 530 mg/g gallic acid equivalent in leaves and from 240 to 520 mg/1 gallic acid equivalent in roots. The total flavonoids content was ranged from 140.870 to 360.750 mg/1 quercetin equivalent in leaves and from 138.678 to 357.670 mg/1 quercetin equivalent in roots. The total tannins contents ranged from 210 to 300 mg/l tannic acid equivalent in leaves and 190 to 270 mg/1 tannic acid equivalent in roots. The study showed significant amount of gallic acid, quercetin and tannic acid equivalents were present in S.Borumensis extracts which may responsible for valuable pharmacological property of the plant.

Keywords: Phytochemical Screening, Stylochiton Borumensis, Total Phenolic Content, Total Flavonoid Content, Total Tannins Content.

Introduction

Since ancient times, natural products obtained from plant sources remains as a major source of preventive and curative items. This result in the large number of population is still dependent on the medicinal plants for their preventive and curvative properties. According to World Health Organization, traditional medicines, including herbal medicine, have been, and continue to be, used in every country around the world in some capacity. In much of the developing world, 70-95% of the population relies on these traditional medicines for primary care [1]. This may be due to better cultural acceptability, compatibility with human body and lesser side effects.

Sudan is regarded as a country rich with indigenous herbal sources. But, unlike China, Sudan has not been able to capitalize its herbal wealth by promoting its use to developed countries despite of renewed interest in herbal medicine as this work requires well documented traditional usage, single, plant medicine, free from pesticide residue, heavy metals etc., standardization based in chemical and activity profile, safety and stability. One such plant we are going to discuss here is *Stylochiton borumensis* which belonging to family Araceae and considered as important medicinal plant of Sudan and some other countries. *S.borumensis* is widely distributed in African countries such as Niger, North Nigeria, Sudan, Tanzania and Mozambique. The plant is grown in wet soil and high rain full savannah [2]. The plant used in traditional medicine for treatment of many diseases, in Tanzania the root and bark is used against convulsion, gonorrhea, bilharzias , heart burn, stomach – ache , constipation and wound and snake bites [3]. In Sudan the roots used to relief the pain of scorpion sting, so that give its local name irrig al agrab or moura. These activities are because of complex mix of phytochemical compounds present in S.borumensis. Hence, the objective of present paper was to investigate the phytoconstitiuents present in leaves and roots of *S.borumensis* and determine total phenols, flavonoids and tannins content for the plant.

Collection and identification of plant materials

Leaves and roots of *S.borumensis* were collected by hand from El Debabat 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; where a herbarium specimen voucher number was U.B.H 5/9/015. After authentification, 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.

The coarsely powdered leaves and 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 material x 100
The extracts were collected and stored at 4° C [4].

Preliminary phytochemical screening

A systematic and complete study for crude drugs includes a complete investigation of both primary and secondary metabolites derived from plant metabolism. Different qualitative test were performed for establishing profiles of various extracts for their nature of chemical composition. The extracts obtained were subjected to following chemical tests for identification of various phytoconstitiuents as methods given by Harborne [5]. There were no previously isolated compounds.

1-Test for Alkaloids

The various extracts were basified with ammonia and extracted with chloroform. The chloroform solution was acidified with dilute hydrochloric acid. The acid layer was used for testing the alkaloids. The following four tests were used for detection of alkaloids

A -Dragendroffs test: (potassium bismuth iodide)
The acid layer was treated with few drops Dragendroffs reagent. Formation of reddish brown precipitate indicated the presence of alkaloid.

B-Wagner's test: (Iodine in potassium iodide)
The acid layer was treated with few drops of Wagner's reagent. Formation of reddish brown precipitate indicated the presence of alkaloid.

C-Mayer's test: (potassium mercuric iodine solution):
The acid layer was treated with few drops of Mayer's reagent. Formation of creamy white precipitate indicated the presence of alkaloids.

D-Hager's test: (One g picric acid in 100 ml distilled water):
The acid layer was treated with few drops of Hager's reagent. Formation of yellow precipitate indicated the presence of alkaloids.

2-Test for flavonoids [6]

The following four tests were used for detection of flavonoids:
A-Shinoda test: to alcoholic solution of extract a few fragments of magnesium ribbon and concentrated hydrochloric acid were added. Appearances of red to pink color after few minutes indicated the presence of flavonoids.

B- Ferric chloride test: few drops of neutral ferric chloride solution were added to little quantity of alcoholic extract. Formation of blackish green color indicated the presence of flavonoids.

C- Lead acetate test: to the extract, few drops of aqueous basic lead acetate solution were added. Formation of yellow precipitate indicated presence of flavonoids.

D- Sodium hydroxide 10% test: to alcoholic solution few drops of Sodium hydroxide was added. Formation of intense yellow color which disappeared after adding diluted hydrochloric acid.

3-Test for sterols / triterpenoid [7,8]

Different extracts were dissolved in chloroform, filtered and filtrate was tested for sterols and triterpenes.

A- Salkowski test: few drops of concentrated sulphuric acid were added to chloroform solution, shaken and allowed to stand until appearance of red color in lower layer indicated the presence of sterols. And appearance of golden yellow color indicated the presence of triterpenes

B- Liberman- Burchard test: to chloroform solution, a few drops of acetic anhydride were added and mixed well. 1 ml of concentrated sulphuric acid was added from the side of the test tube, appearance of reddish brown ring indicated the presence of sterols. And appearance of deep red color indicated the presence of triterpenes.

4- Test for tannins [9]

The following two tests were used for detection of tannins
A-Ferric chloride test: to extracts a few drops of 1% neutral ferric chloride solution was added. Formation of blackish blue color indicated the presence of tannins.

B-Gelatin: test to extracts 1% solution of gelatin were added to solution containing 10% sodium chloride. Formation of white precipitate indicated presence of tannins.

5-Test for saponins [8]

A- Foam test: small amount of extract was shaken with little quantity of water, if foam produce persist for 10 minutes, it indicated presence of saponin.

6- Test for Coumarin

One gram of powdered plant kept with distilled water in a test tube, covered with paper soaked in sodium hydroxide was diluted and boiled. Yellow fluorescence under ultra-violet lamp indicated presence of coumarins.

7-Test for Polyphenol

A- Ferric chloride 5%: Small amount of extract dissolved in water or mixed with alcohol, few drops of 5% ferric chloride was added. Formation of red, blue, green or purple colors indicated presence of phenolic compounds.

8-Test for Carbohydrates

Small amount of extracts/ fractions were dissolves in little quantity of distilled water and filtered separately. The filtrates were used to test presence of carbohydrates.

A- Molisch's test: (general test for the presence of carbohydrate):
To 1 ml of diluted extracts 2 drops of α-napthol was added and 2ml concentrated sulphuric acid was poured carefully down the side of the test tube. Formation of purple ring at interface of two layers indicated presence of carbohydrates.

B- Benedict's test: (for reducing monosaccharide):
To 1 ml of extract 1ml of Benedict's reagent was added then boiled in water bath for 5 minutes. Formation of orange or brick-red solution with precipitate indicated presence of reducing sugars.

C- Fehling's test: (for reducing monosaccharide)
To 1m of extract 1ml of Fehling's reagent was added then boiled in water bath for 5 minutes. Formation of green, yellow, orange and brick-red solution with precipitate indicated presence of reducing sugars.

D- Bial's test: (for pentose sugar) To 1 ml of bial's reagent 1 ml of extract was added then boiled in water bath for 3 minutes. Formation of blue-green solution indicated presence of pentose sugars.
E- Seliwanoffs test: (for ketose sugar) To 1 ml of Seliwanoff's reagent 1 ml of extract was added then boiled in water bath for 5 minutes .Formation of deep cherry red color indicated presence of ketose sugars.

9- Test for Amino acids and Protein
A- Ninhydrine test: (general test for the presence of amino acid) To 1 ml of extract added 0.5 ml Ninhydrine reagent and heated in boiling water bath for 2 minutes .Formation of blue-purple color solution indicated presence of free amino acid.

B- Biuret test: (general test for the presence of protein) To 1 ml of extract added 1 ml of Biuret reagent formation of purple color indicated presence of protein.

Estimation of total phenols content (Folin- Ciocalteau assay)
The total phenolic content was determined by Folin-Ciocalteau reagent (F-C) method as described by Shivakumar [10]. 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.

Estimation of total flavonoids content
Total flavonoids content was determined following a method described by Shivakumar [10]. In 10 ml test tube, 0.3 ml of extracts, 3.4 ml of 30% methanol, 0.15 ml of sodium nitrite (0.5 M) and 0.15 0f aluminum chlorides (0.3 M) were mixed. After 5 minutes, 1 ml of Sodium hydroxide (1M) was added. The solution was mixed well and the absorbance was measured against the reagent blank at 506 nm. Total flavonoids content was expressed as quercetin (mg/1) using the following equation based on the calibration curve $Y = 0.0007x+ 0.053$ where Y was the absorbance. Calibration curve was constructed, using quercetin (50-800 mg/1) as Standard. Total flavonoids content in the plant extracts was calculated using formula: Total flavonoids content= Q x V/m , where Q is the Querectin equivalence (mg/ml) or concentration of quercetin established from calibration curve; V is the volume of extracts in ml and m is weight (g) of pure plant extracts.

Estimation of total tannins content
The total tannins content was determined by a method as described by Shivakumar [10]. with some modifications. About 1 ml of extract (1 mg/ml) was transferred to test tube, 1 ml of 1% potassium ferric cynide and 1ml of ferric chloride were added, and the volume was made up to 10 ml with distilled water. Absorbance was measured at 510 nm against a reagent blank. The total tannins content was calculated using the following equation $Y = 0.0009 x + 0.043$ where x= concentration of tannic acid (mg/1) corresponding to optical density. A calibration curve was constructed, using tannic acid (100 – 900 mg/g) as standard. Total tannins contents of the extracts (mg/1) expressed as tannic acid equivalents and calculated using formula: Total tannins content = TA x V/m, where TA is the tannic acid equivalence (mg/ml) or concentration of tannic acid established from the calibration curve, V is the volume of extracts in ml and m is the weight (g) of the pure plant extract.

Results and Discussion

Preliminary phytochemical screening
The results of general phytochemical screening of *Stylochiton borumensis* leaves as reported in (table 1) showed high amount of alkaloids in all extracts. High amount of flavonoids was found in ethanol 80% and distilled water extracts but not detected in petroleum ether and chloroform extracts. High to moderate amount of tannins detected in all extracts. High amount of polyphenolic compounds was found in ethanol 80% and distilled water extracts, moderate amount was found in chloroform extracts and low amount present in petroleum ether. Low to moderate amount of sterol and triterpenes were found in all extracts. High amount of coumarin was found in petroleum ether and chloroform extracts, but low amount was found in ethanol 80% and distilled water extracts. These finding in general agree with some results reported by Missa, M. [11].

Results of general phytochemical screening activity of *Stylochiton borumensis* roots extracts as presented in (table 2) showed high amount of alkaloids in all extracts. High amount of flavonoids was found in ethanol 80% and distilled water extracts, but low amount of flavonoids was detected in petroleum ether and chloroform extracts. Low amount of saponin was found in ethanol 80% and distilled water extracts, but not detected in petroleum ether and chloroform extracts. Moderate amount of tannins was found in all extracts. High amount of phenolic compounds was found in ethanol 80% and distilled water extracts and moderate amount was detected in petroleum ether and chloroform extracts. Low amount of sterol was found in petroleum ether extract, but not detected in the rest of other three extracts. High amount of triterpenes was detected in all four extracts. High amount of coumarin was found in petroleum and chloroform extracts, but low amount of coumarin was detected in ethanol 80% and distilled water extracts. These finding in general agree with some results reported by El Ghazali [12].

Total phenolic, flavonoids and tannins contents
The total phenolic, flavonoids and tannins contents of leaves and roots extracts of S. borumensis were evaluated and results are presented in Table (3). The total phenolic content ranged from 460 to 530 mg/1gallic acid equivalent in leaves and from 240 to 520 mg/l gallic acid equivalent in roots. The highest content was found in the ethanol extract (leaves = 510 mg/l, roots = 500 mg/l) followed by the chloroform (leaves = 480 mg/l, roots = 440 mg/l), petroleum ether extracts (leaves = 460 mg/l, roots = 420 mg/l) and distilled water extracts (leaves = 380 mg/l, roots = 340 mg/l) respectively.

Tannins content was calculated as tannic acid equivalent ranged from 210 to 300 mg/l tannic acid equivalent and from in leaves and190 to 270 mg/l in roots. The highest content was found in the ethanol extract (leaves = 300 mg/l, roots = 270 mg/l) followed by the distilled water extracts (leaves = 210 mg/l, roots = 190 mg/l).

Flavonoids content was calculated as quercetin equivalent ranged from 140.870 to 360.750 mg/l quercetin acid equivalent in leaves and from 138.678 to 357.670 mg/l in roots. The highest content was found in the ethanol extract (leaves = 360.750 mg/l, roots = 357.670 mg/l) followed by the chloroform (leaves = 230.960 mg/l, roots = 225.670 mg/l), petroleum ether extracts (leaves = 220.340 mg/l, roots = 210.450 mg/l) and distilled water extracts (leaves = 140.870 mg/l, roots = 138.678 mg/l) respectively. Flavonoids and phenols are considered as anti-capillary fragility and anticancer compound [13].

Polyphenol have inhibitory effect on mutagenesis and carcinogenesis in human when ingested in daily diet [14]. As these compounds are responsible for antioxidant activity of plant material further it is required to perform in-vitro antioxidant activity using various models that can establish a good correlation between the data obtained in present experiment. Future prospects include isolation of flavonoids and phenols responsible for the activity and determining structure.

Table 1: Preliminary phytochemical screening of *S.borumensis* leaves extracts

Secondary metabolites	Test reagent	Method of extraction			
		P.E	ChCl3	EtOH 80%	D. w
Alkaloids	Dragendroffs	+	+	+	+
	Mayers	+	+	+	+
	Hager's	+	+	+	+
	Wagner's	+	+	+	+
Flavonoids	Mg/H_2SO_4 conc.	-	-	-	-
	Aluminum chloride	-	-	+	+
	Lead acetate	-	-	+	+
	Sodium chloride 10%	-	-	+	+
Saponin	Foam test	-	-	+	+
Sterol	Liberman's	+	+	+	-
	Salkowski	+	+	+	-
Phenol	Ferric chloride	+	+	+	+
Triterpenes	Liberman's	+	+	+	+
	Salkowski	+	+	+	-
Tannins	Ferric chloride	+	+	+	+
	Gelatin	+	+	+	+
Coumarin	Potassium Chloride/UV	+	+	+	+
Carbohydrates	Molisch	+	+	+	+
	Benedicts	+	+	+	+
	Fehling	-	+	+	+
	Bials	_	+	+	+
	Seliwanoffs	-	-	+	-
Protein	Biuret	-	-	+	+
Amino acid	Ninhydrine	-	-	+	+

Table 2: Preliminary phytochemical screening of *S.borumensis* roots extracts

Secondary metabolites	Test reagent	Method of extraction			
		P.E	ChCl3	EtOH 80%	D. w
Alkaloids	Dragendroffs	-	+	+	+
	Mayers	+	+	+	+
	Hager's	+	+	+	+
	Wagner's	+	+	+	+
Flavonoids	Mg/H_2SO_4 conc.	-	-	-	-
	Aluminum chloride	-	-	-	-
	Lead acetate	-	-	+	+
	Sodium chloride 10%	+	+	+	+
Saponin	Foam test	-	-	+	+
Sterol	Liberman's	-	-	-	-
	Salkowski	+	+	+	+
Phenol	Ferric chloride	+	+	+	+
Triterpenes	Liberman's	+	-	-	-
	Salkowski	+	-	-	-
Tannins	Ferric chloride	+	+	+	+
	Gelatin	+	+	+	+
Coumarin	Potassium Chloride/UV	+	+	+	+
Carbohydrates	Molisch	+	+	+	+
	Benedicts	-	+	+	-
	Fehling	-	+	+	-
	Bials	-	+	+	-
	Seliwanoffs	-	-	-	+
Protein	Biuret	-	-	+	+
Amino acid	Ninhydrine	-	-	+	+

Note: (+) presence; (-) absence
P.E = petroleum ether (40-60 °C); Chcl3= chloroform; EtOH 80% = Ethanol 80 %

Table 3: Concentration (Mean) of total phenols, tannins and flavonoids contents of *Stylochiton borumensis* leaves and roots extracts

Content	Part of plant	P.E Mg/1	$ChCl_3$ Mg/1	EtOH 80% Mg/1	D. w Mg/1
Phenols	Leaves	460	480	510	380
	Roots	420	440	500	340
Tannins	Leaves			300	210
	Roots			270	170
Flavonoids	Leaves	220.340	230.960	360.750	140.870
	Roots	210.450	225.670	357.670	138.678

Conclusion

Stylochiton borumensis contain considered amount of phenolic and flavonoids compound which may be responsible for valuable pharmacological activities. Phenolics and flavonoids compound are responsible for antioxidant, anticancer and anti-capillary

fragility. The presence of these compounds in the *S.borumensis* leaves and roots shows the medicinal importance of the plant. Further the plant could be considered for antioxidant, anticancer and immunomodulatory activity.

References

1. Robonson M M, Zhang X (2011) the world medicine situation, traditional medicine: global situation, issues and challenges, 3rd edition, WHO, Geneva.
2. El Ghazali G E B, El Tohami M S, El Egami A A B, (1994) Medicinal plants of Sudan, Medicinal plant of the Blue Nile province. National Centre for Research, Medicinal and Aromatic plant Research Institute, Khartoum 3: 26-27.
3. Galadima M (2008) Plant species and their uses. Convention Biology Diversit. 257. GBIF (2011) Royal Botanic Gardens, Kew http:// www.gbif.org.
4. Lohar DR (2010) Protocol for testing of Ayurvedic, Siddha and Unani medicines, pharmacopeia laboratory for Indian medicines, Ministry of Health and Family Welfare, Ghaziabad 110-11.
5. Harborne J B (2007) phytochemical methods: A guide to modern techniques of plant analysis, 3rd edition, Chapman and Hall, London 75-125.
6. Peach K, Tracey M V (1956) Modern methods of plant analysis, Vol. 13, Springer Verlag Berlin 27-125.
7. Gibbs R D (1974) Chemotaxonomy of flowering plants, Vol. 1, McGill Queen's University Press, Montreal and London.
8. Kolkate C K (1994) Practical Pharmacognosy, 4th edition, Vallabh Prakashan, New Delhi, India 124-125.
9. Sofowora A (1993) Medicinal plants and traditional medicine in Africa – Chichester John Wiley & sons, New York 97-145.
10. Shivakumar B S, Ramaiah M, Hema M R, Vijay Kumar M, Vaidya V.P (2012) Quantitative determination of total content of phenol, flavonoid and tannin in leaf extract of Barlaria Biocifolia LAMT Pharmacy and Technology Research 2(5)-422.
11. Missa M S A (2016) Isolation and Identification of some Sudanese Medicinal Plants. PhD. Thesis-University of Sudan for Science and Technology.
12. El Ghazali G E M, El Tohami (1997) Medicinal plants of the Sudan part IV. Medicinal plants of Northern Kordofan, Khartoum -National Centre for Research.
13. Parmar N S, Ghosh M N (1977) Indian J Exp Biol 15: 311-313.
14. Halliwell B (1994) Lancet 344: 721-724.