Evaluation of Phytochemical Constituents and Some Biological Potency of the Root of *Streptocaulon tomentosum* Wight and Arn (Myinsa-gonni) and the Aerial Parts of *Bacopa monnieri* (L) Wettst (Byone-hmwe)

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Abstract

The present study examines the phytochemical constituents and some biological activities of the root of *S. tomentosum* and the aerial parts of *B. monnieri.* The presence of tannins, alkaloids, phenolic compounds, terpenoids, flavonoids, α-amino acids, saponins, carbohydrates, steroids, organic acids, and glycosides was confirmed by the qualitative phytochemical screening of the powders from two plants. The Folin-Ciocalteu test was used to assess the total phenolic content, and the aluminum chloride colorimetric assay was used to estimate the total flavonoid content. The DPPH assay was used to test two samples' ethanol and watery extracts for antioxidant activity. The cytotoxic potential of the brine shrimp lethality test was examined. The aerial parts of *B. monnieri* and the root of *S. tomentosum* had 50 % lethality concentrations of ethanol extract with LC_{50} values of 400 µg/mL and 406 µg/mL, and LC_{50} values of watery extract (200 µg/mL and 382 µg/mL) were also evaluated. These assays suggested that the ethanol extract (IC_{50} = 470 µg/mL, 669.6 µg/mL) had more potent antioxidant activity than the watery extract (IC_{50} = 770 µg/mL, 901.01 µg/mL). This study's results concluded that the two different types of extracts contained significant potential for therapeutic applications.

***Keywords*:** *Streptocaulon tomentosum*, *Bacopa monnieri*, phytochemical, antioxidant and cytotoxic potential

Introduction

Streptocaulon tomentosum Wight & Arn. (Asclepiadaceae) is a liana widely distributed in Myanmar, China, Vietnam, and neighboring countries. The roots of *Streptocaulon tomentosum* are used in the traditional medicine of Myanmar for the treatment of cancer, dysentery, and stomachache, whereas the leaves are used externally for the treatment of snake poisoning. (Luay *et al*., 2011). *Bacopa monnieri* (Scrophulariaceae) is a small creeping, spreading, a succulent herb with numerous branches and small fleshy, oblong leaves. Flowers and fruits appear in summer (Chopra *et al*., 1956). In traditional medicine, it is used to treat various nervous disorders, digestive aid, improve learning, and memory, and provide relief to patients with anxiety, and skin disorders; specific uses include the treatment of asthma, insanity, and epilepsy (Kamkaew *et al*., 2013). This plant has been chosen because of its various pharmaceutical and medicinal properties. Since the important elemental constituents, phases, and complexes of the medicinal plant possess the different curative capabilities of human disease (Beheraa *et al.*, 2016). The present study was to determine the phytochemical composition of extracts and to screen the antioxidant and cytotoxic activities of the two plants.

(a)

(b)

Figure 1 Photographs of (a) the root of *S. tomentosum* and (b) the aerial parts of *B. monnieri*

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Materials and Methods

Collection of plant materials

Two herbal plants were selected to be used in the study dried: the root of *Streptocaulon tomentosum* Wight & Arn (Myinsa-gonni) was collected from Launglon Township, Dawei District, Tanintharyi Region in August 2019, and the aerial parts of *Bacopa monniera* (L) Wettst (Byone-hmwe) was collected from Twantay Township, Yangon Region in July 2019. Both plants were identified with scientific names by the authorized botanists at the department of Botany, University of Yangon. The two samples were washed thoroughly, initially with running tap water and then with double distilled water to remove any debris or dust particles. Furthermore, the two samples were shade dried for 3-4 days at room temperature. After drying, the leaves were ground into a fine powder using an electric blender.

Qualitative Screening of the Phytochemicals

In order to know, the types of Phyto-organic constituents present in the root of *S. tomentosum* and the aerial parts of *B. monnieri*, a preliminary phytochemical investigation of samples was carried out according to the conventional methods (Robinson, 1983).

Determination of Total Phenol Contents by Folin- Ciocalteu method

The folin-Ciocalteu method was carried out to determine the total phenol content of the root of *S. tomentosum* and the aerial parts of *B. monnieri* in ethanol and watery extracts using gallic acid as a standard (Ghafari *et al*., 2014). Each plant extract (1000 µg/mL) of 1mL was mixed with 5mL of Folin-Ciocalteu reagent in a test tube and shaken. Then 4 mL of saturated sodium carbonate (1 M) was added to the mixture. Then the tubes were allowed to stand for 30 min at room temperature, and the absorbance of the resulting blue solution was recorded at 765 nm using a UV-visible spectrophotometer. A standard curve was prepared by mixing methanol solution of gallic acid (100, 50, 25, 12.5 and 6.25 µg/mL) as shown in the above procedure instead of the sample solution. The experiment was done in triplicate. The total phenolic content was expressed as mg of gallic acid equivalents.

Determination of Total Flavonoid Contents by aluminium Chloride Colorimetric assay

The total flavonoid content (TFC) of the root of *S. tomentosum* and the aerial parts of *B. monnieri* extracts was estimated using the aluminium chloride colorimetric assay method (Oyedemi *et al*., 2010). Each plant extract (1000 µg/mL) of 1 mL was mixed with 3 mL of methanol, 0.1 mL of 1 % aluminum chloride solution, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The resultant mixture was allowed to stand for 30 min at room temperature. The absorbance of the resulting yellow solution was recorded at 415 nm using a UV-visible spectrophotometer. A standard curve was prepared with the quercetin solution (100, 50, 25, 12.5 and 6.25 µg/mL) as shown in the above procedure instead of the sample solution. The experiment was done in triplicate. The total flavonoid content was expressed as mg quercetin equivalents QE per g of dry sample.

Determination of antioxidant activity

The free radical scavenging activity of ethanol and watery extracts of two samples was evaluated by the DPPH radical scavenging assay using a UV visible spectroscopic method (Ashokkumar and Ramaswamy, 2013). The control solution was prepared by mixing 1.5 mL of 120 µM DPPH solution and 1.5 mL of ethanol in the brown bottle. The sample solution was also prepared by mixing 1.5 mL 120 µM DPPH solution (Absorbance = 0.8941, used for control) and 1.5 mL of test sample solution (1000, 500, 250, 125 and 62.5 µg/mL). These bottles were incubated at room temperature and were taken off the shaker for 30 min. After 30 min, the absorbances of these solutions were measured at 517 nm using a

UV-7504 Spectrophotometer. Absorbance measurements were done three times for each concentration, and a mean value was obtained. The percentage of radical scavenging activity (% RSA) was calculated by the following equation.

$$\% \text{ RSA} = [A_{DPPH} - (A_{\text{Sample}} - A_{\text{blank}}) / A_{DPPH}] \times 100$$

Where, % RSA = % radical scavenging activity

A_{DPPH} = absorbance of DPPH in EtOH solution

A_{Sample} = absorbance of sample and DPPH solution.

A_{blank} = absorbance of sample and EtOH solution

Cytotoxicity Effects by Brine Shrimp Lethality Bioassay

The brine shrimp lethality bioassay was used to predict the cytotoxic potency of ethanol and watery extracts of the root of *S. tomentosum* and the aerial parts of *B. monnieri* according to the procedure described by (Olowa *et al*., 2013) The brine shrimp (Artemia salina) was used in this study of cytotoxicity bioassay (Ali *et al.,* 2013). Artificial seawater (9 mL) and (1 mL) of different concentrations of samples and standard solutions such as potassium dichromate, and caffeine were added to each test tube. Alive brine shrimps (10 nauplii) were then taken with a Pasteur pipette and placed into a test tube. They were incubated at room temperature for about 24 h. After 24 h, the number of dead or survival brine shrimps was counted and 50 % lethality concentration (IC_{50}) was calculated (Sahgal *et al*., 2010). The control solution was prepared as the above procedure by using distilled water instead of sample solution The cytotoxicity of test extracts was expressed according to Deciga-Campos criteria that the toxicity for the assessment of plant extracts was classified with LC_{50} values above 1000 µg/ml are non-toxic, between 500 and 1000 µg/mL are weak toxic, and that below 500 µg/ml are toxic (Deciga-Campos *et al*., 2007).

Result and Discussion

Preliminary Phytoconstituents Present in the Crude Extracts of the Root of *S. tomentosum* and the Aerial Parts of *B. monnieri*

The preliminary screening of phytochemicals in the root of *S. tomentosum* and the aerial parts of *B. monnieri* was done by conventional methods. The phytochemical tests revealed the presence of secondary metabolites such as alkaloids, α-amino acids, carbohydrates, glycosides, saponins, phenolic compounds, flavonoids, tannins, steroids, and terpenoids, but cyanogenic glycosides were not observed in both samples. Besides, reducing sugars and starch was found in the roots of *S. tomentosum*.

Total Phenolic and Flavonoid Contents of Crude Extracts of the Root of *S. tomentosum* and the Aerial Parts of *B. monnieri*

Phenolic compounds include phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, and quinines. Total phenolic and total flavonoid content were determined by a linear regression equation respectively. The total phenol and flavonoid contents of the two plants are shown in Table 1. The ethanol extract of the root of *S. tomentosum* and the aerial parts of *B. monnieri* had (22.16 ± 0.018 mgGAE/g extract), (7.44 ± 0.029 mgGAE/g) total phenolic contents, and (33.1± 0.005 mgQE/g extract), (24.05±0.04 mgQE/g) total flavonoid contents. According to these results, the ethanol extract of the two plants had higher total phenol and flavonoid contents than that of the water extract.

Table 1 Estimation of Total Phenolic and Flavonoid contents of the Root of *S. tomentosum* and the Aerial Parts of *B. monnieri*

TPC and TFC content	*S. tomentosum*		*B. monnieri*	
	Ethanol extract	**Watery extract**	**Ethanol extract**	**Watery extract**
Total phenolic content (mg GAE / g)	22.16 ± 0.018	4.61 ± 0.008	7.44 ± 0.029	3.58 ± 0.007
Total flavonoids content (mg QE / g)	33.10 ± 0.005	32.41 ± 0.004	24.05 ± 0.004	15.23 ± 0.002

In Vitro Antioxidant Assay

The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple colour). As a consequence, the absorbance of the DPPH decreases when antioxidants react with DPPH, which is a stable free radical that becomes paired off in the presence of a hydrogen donor and is reduced to the DPPH. Radical to the DPPH-H form, results in decolorization (yellow colour) with respect to the number of electrons are captured. More decolorization is the ability to reduce (Ghosh, 2011). The determination of radical scavenging activity by the DPPH method based on the change in absorbance of crude extract solution in various concentrations. A decreased absorbance exhibits an increase in radical scavenging activity. The radical scavenging activity of crude extracts was expressed in terms of % RSA and IC_{50} (50 % inhibitory concentration). Since the lower the IC_{50} values, the higher the antioxidant activity of the sample occurs. From the antioxidant activity data, the IC_{50} value of ethanol extract of the root of *S. tomentosum* and the aerial parts of *B. monnieri* (470 µg/mL, 669.6 µg/mL) had higher antioxidant activity than the watery extract of the root of *S. tomentosum* and the aerial parts of *B. monnieri* (770 µg/mL, 901.01 µg/mL, respectively). The antioxidant activity is compared with standard ascorbic acid (IC_{50} = 11.77 µg/mL) (Table 2, 3, and figure 1).

Table 2 Average % Radical Scavenging Activity and IC_{50} Values of Crude Extracts from the Root of *S. tomentosum* and the Aerial Parts of *B. monnieri*

Extracts	% RSA ± SD at Different on centration (µg/mL)					IC_{50} (µg/mL)
	62.5	**125**	**250**	**500**	**1000**	
B. monnieri (watery extract)	11.36 ± 0.33	20.13 ± 1.12	30.13 ± 0.56	40.38 ± 0.48	58.02 ± 0.37	770.54
B. monnieri (ethanol extract)	25.20 ± 0.57	35.75 ± 0.98	46.30 ± 0.66	57.30 ± 0.33	67.14 ± 0.26	470.15
S. tomentosum (watery extract)	13.46 ± 0.42	20.30 ± 1.10	29.70 ± 1.40	39.85 ± 0.98	50.70 ± 0.02	901.01
S. tomentosum (ethanol extract)	17.60 ± 0.33	24.06 ± 0.02	32.40 ± 0.46	45.02 ± 0.02	63.87 ± 0.03	669.60

Table 3 % RSA (Radical Scavenging Activity) of Standard Ascorbic Acid

Sample	% RSA ±SD at different concentrations (µg/mL)					IC 50 (µg/mL)
	6.25	12.5	25	50	100	
Ascorbic acid	26.90 ± 0.04	52.41 ± 0.01	70.55 ± 0.01	88.34 ± 0.01	94.62 ± 0.01	11.90

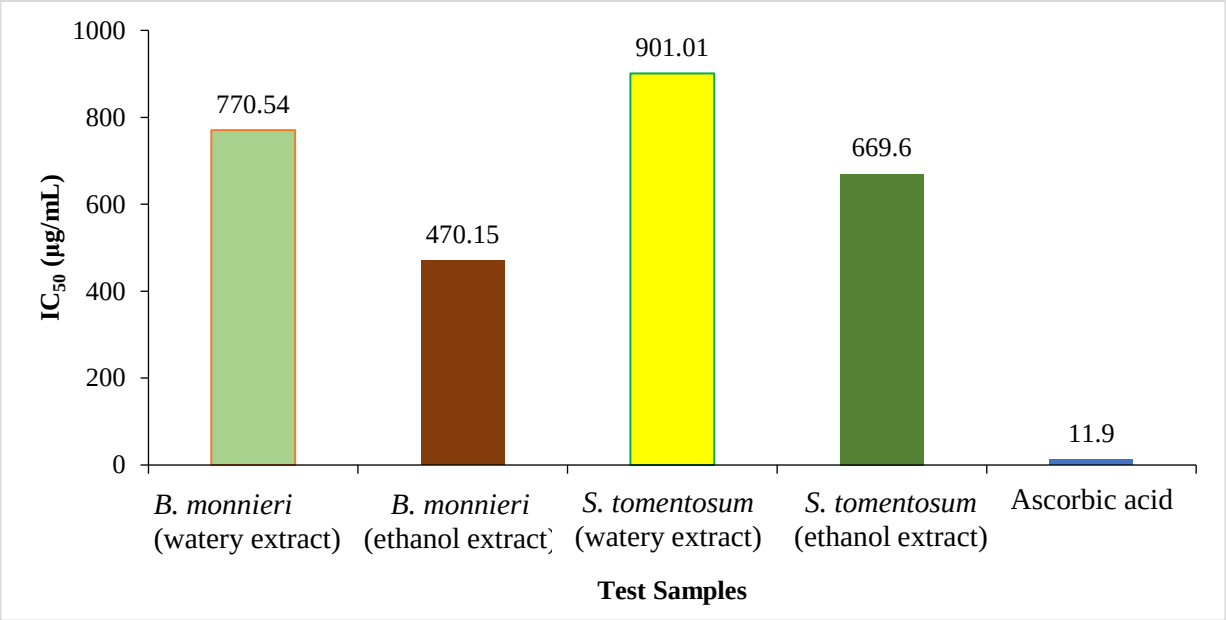

Figure 2 A bar graph of IC_{50} values of crude extracts from the root of *S. tomentosum* and the aerial parts of *B. monnieri*

Cytotoxicity Effect of Crude Extracts of the Root of *S. tomentosum* and the Aerial Parts of *B. monnieri*

Most toxicity studies that use the Brine Shrimp Assay determine the toxicity by counting the survived nauplii after 24 h of exposure to the test. The cytotoxicity activity of ethanol and watery extracts of the root of *S. tomentosum* and the aerial parts of *B. monnieri* was evaluated by the brine shrimp lethality bioassay. The cytotoxic effect was expressed as LC_{50} values (50 % Lethality Concentration). Standard Potassium dichromate ($K_2Cr_2O_7$) and caffeine were chosen because $K_2Cr_2O_7$ is a well-known toxic agent in this assay and caffeine is a natural product. The obtained results of the ethanol and watery extracts of the root of *S. tomentosum* and the aerial parts of *B. monnieri* are reported in table 3 and figure 5. From the results, the LC_{50} values of ethanol and watery extracts of the roots of *S. tomentosum* (400 µg/mL and 200 µg/mL) and the aerial parts of *B. monnieri* were (406 µg/mL and 382 µg/ mL), respectively. For the standard $K_2Cr_2O_7$ was 56.44 µg/mL and caffeine was > 1000 µg/mL. The resulting LC_{50} values were compared with the Deciga-Campos criteria. The ethanol and watery extracts of the root of *S. tomentosum* and the aerial parts of *B. monnieri* showed significant toxicity due to LC_{50} values below 500 µg/mL.

Table 4 Cytotoxicity of Ethanol and Watery Extracts from the Root of *S. tomentosum* and the Aerial Parts of *B.monnieri*

Test Sample	% Mortality of Brine Shrimp Concentration ± SD in Different Concentration of Sample (µg/mL)					LC_{50} (µg/mL)
	50	100	200	400	800	
B. monnieri (watery extract)	36.67 ± 0.58	43.34 ± 0.55	46.67 ± 0.58	53.33 ± 0.58	60.00 ± 0.00	382
B. monnieri (ethanol extract)	20.00 ± 0.00	30.00 ± 0.00	43.34 ±0.58	56.67 ± 0.58	70.00 ± 0.00	406
S. tomentosum (watery extract)	33.34± 0.58	43.34 ± 0.58	50.00 ± 0.00	60.00 ± 1.00	66.70 ± 1.15	200
S. tomentosum (ethanol extract)	26.67 ± 0.53	36.67 ± 0.58	43.34 ± 0.58	50.00 ± 0.00	63.34 ± 1.28	400
* patassium dichromate	36.67 ± 0.53	53.34 ± 0.58	70.00 ± 1.00	86.67 ± 1.15	100.00±0.00	56..44
* Caffeine	0.00 ± 0.00	0.00 ± 0.00	6.67 ± 1.53	16.67 ± 0.58	23.34 ± 1.53	>1000

*Used as Cytotoxic standard

Conclusion

In this research work, the following inferences could be deduced: the preliminary phytochemical analysis revealed the presence of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, steroids, tannins, and terpenoids in the root of *S. tomentosum* and the aerial parts of *B. monnieri*. Moreover, cyanogenic glycosides were absent in both plant samples. Besides, starch and reducing sugars were not found in the aerial parts of *B. monnieri*. The root of *S. tomentosum* and the aerial parts of *B. monnieri* significantly showed total phenolic and flavonoid content. In the brine shrimp lethality bioassay, the results showed that ethanol and watery extracts of *S. tomentosum* and *B. monnieri* possessed a significant cytotoxic effect (LC_{50} below 500 µg/mL) that may be used in traditional medicine formulations to treat many kinds of diseases.

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