SCREENING ON SOME BIOACTIVITIES FROM THE LEAF OF *BAUHINIA PURPUREA* L. (SWEDAW-NI)

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Abstract

In the present study, the leaf of *Bauhinia purpurea* L., locally known as "Swedaw-ni" was selected to screen some bioactivities. Firstly, the phytochemical constituents were investigated by the reported chemical methods. The qualitative elemental analysis was done by EDXRF technique. The crude extracts were prepared by the solvent extraction method. Total phenolic content in ethanol and watery extracts was carried out spectrophotometrically using Folin-Ciocalteu reagent. Ethanol extract (32.58 µg GAE/mg of extract) showed higher phenolic content than watery extract (21.73 µg GAE/mg of extract). In the study of the antioxidant activity, ethanol and watery extracts were screened by DPPH radical scavenging assay. The ethanol extract (IC_{50} = 5.75 µg/mL) was found to be more potent than the watery extract (IC_{50} = 8.51 µg/mL) in the antioxidant activity. The antimicrobial activity of both extracts was investigated against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *E. coli*. The results showed that all tested microorganisms were susceptible to both ethanol and watery extracts of *B. purpurea* leaf. In addition, acute toxicity of ethanol extract was investigated with the dosage of 300 mg/kg, 2000 mg/kg and 5000 mg/kg body weight on albino mice and no lethality was observed up to fourteen days after administration. The Chemical nature for not been studied yet, however, the leaf of *B. purpurea* is likely to have potent biological activities to use as ingredient in preventive medicine.

Keywords: *Bauhinia purpurea* L., EDXRF, total phenolic content, antioxidant activity, antimicrobial activity, acute toxicity

Introduction

Nature has provided a complete storehouse of remedies to cure ailment of mankind. Herbal medicine, as the major remedy in traditional medical systems, has been used in medical practice for thousands of years and has made a great contribution to maintaining human health. A majority of the world's population in developing countries still relies on herbal medicine to meet its health needs (Kumar *et al*., 2008). *Bauhinia purpurea* L (swedaw-ni in Myanmar) is belonging to the family leguminosae and it is a small to medium-sized deciduous tree. It is native to Southern and Southeastern Asia. It has been planted as an ornamental in many tropical and subtropical regions of the world. The plant is widely distributed throughout Myanmar. It contains major class of secondary metabolites which are flavonoids, glycosides, saponins, triterpenoids, phenolic compounds, oxepins, fatty acids and phytosterols (Kumar and Chandrashekar, 2011). The research has focused on screening of some bioactivities from the leaf of *B. purpurea* (Swedaw-ni). The plant *B. purpurea* is used in several ways for the treatment of skin diseases, wounds, ulcers, cough, dysentery, snakebite, tumors, flatulence, indigestion, piles and also lots of other ailments (Gupta *et al*., 2012). It reported to exhibit various pharmacological activities such as antioxidant activity, hepatoprotective activity, hypoglycaemic activity and antiproliferative activity (Shajiselvin *et al*., 2011). The leaves are used for the treatment of catarrh, infection of children, boil, glandular and swelling (Avinash *et al*., 2011). Its flowers are used to reduce fever. The bark of the plant is used as an astringent and its decoctions are recommended for ulcers as a useful wash solution. The decoction of stem bark orally twice a day is very effective in asthma and other respiratory disorder as an anti-inflammatory agent (Patil *et al*., 2008). The whole plant is used in dropsy, pain, rheumatism, convulsions, delirium and septicemia. The medicinal activities of *B. purpurea* are of great potential and it can be used as drugs of choices. The photographs of leaves and flowers of *B. purpurea* are described in Figure 1.

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(a) (b)

Figure 1 Photographs of *B. purpurea* (a) leaves (b) flowers

Materials and Methods

The chemicals used in this research were obtained from British Drug House (BDH) and the reagents used were analar grade. The instruments used were EDXRF (Shimadzu EDX-7000 spectrometer) and UV - visible Spectrophotometer (Shimadzu UV-240).

Collection and Identification of Plant Material

The fresh leaf of the *B. purpurea* (Swedaw-ni) was collected from the area of Mingalardon Township, Yangon Region, in January 2019. After collection, the sample was identified at Botany Department, Taungoo University. The sample was washed with water to remove adhering dirt and then cut into small pieces, dried at room temperature for one week. The dried sample was ground into powder form with help of a grinding machine and stored in airtight container for further use.

Phytochemical Screening of Leaf of *B. purpurea*

In order to find out the types of phytoconstituents present in sample, phytochemical investigation was carried out by chemical methods (Harborne, 1984; Robinson, 1983).

Qualitative Elemental Analysis of Leaf of *B. purpurea*

For this measurement, pellet of the sample was first made. Energy disperse X-ray fluorescence spectrometer (Shimadzu EDX-7000) can analyze the elements from Na to U under vacuum condition. The individual element in sample is detected by using semiconductor that permits multi-elements, simultaneous analysis. In this way, EDX-7000 spectrometer determines elements that are present in the sample. The elemental contents in *B. purpurea* leaf were determined by EDXRF spectrometer at Taungoo University.

Preparation of Crude Extracts of Leaf of *B. purpurea*

Ethanol crude extract was prepared from 100 g of dried powdered sample mixed with 400 mL of ethanol and kept for three days. The mixture was then filtered and evaporated to dryness under reduce pressure using a rotatory evaporator to produce the yield.

Watery crude extract was also prepared from 100 g of dried powdered sample mixed with 400 mL of distilled water and boiled for 2 h. The mixture was then filtered and evaporated to dryness on water bath to produce the yield. Each extract was stored in a desiccator containing dry silica gel prior using in each experiment.

Determination of Total Phenolic Content of Leaf of *B. purpurea*

The total phenolic content in ethanol and watery extracts of *B. purpurea* leaf was determined by using Folin-Ciocalteu colourimetric method described by Kim *et al*., (2003).

In this procedure, 1 mL of tested sample solution (1000 µg/mL) was added to 5 mL of 10 % FC reagent and incubated at room temperature for 30 min. Then, 4 mL of 1 M sodium carbonate solution was added to the mixture and the tubes were kept at room temperature for 15 min. The absorbance of reaction mixture was measured at λ_{max} 765 nm using UV-visible spectrophotometer. The standard calibration curve was constructed by plotting the absorbance vs different concentrations (100, 50, 25, 12.5, 6.25 and 3.125 µg/mL) of gallic acid. Total phenolic content was expressed as microgram of gallic acid equivalent per milligram of crude extract (µg GAE/mg of extract).

Screening of some Bioactivities of Leaf of *B. purpurea*

Antioxidant activity screening

The antioxidant activity of ethanol and watery extracts of *B. purpurea* leaf was determined by DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging assay using UV-visible spectrophotometer (Jain *et al*., 2008). In this procedure, 1.5 mL of tested sample solution with six different concentrations (50, 25, 12.5, 6.25, 3.125 and 1.5625 µg/mL) was mixed with 1.5 mL of DPPH solution (60 µM). The absorbance values of these solutions were measured by UV-visible spectrophotometer at 517 nm at 30 min intervals against a blank solution. Absorbance was measured in triplicate for each solution and the mean values so obtained were used to calculate the percent inhibition of oxidation by the following equation:

$$\% \text{ inhibition} = \frac{\text{Control - (Sample - Blank)}}{\text{Control}} \times 100$$

Control = the absorbance of DPPH in EtOH solution

Sample = the absorbance of sample and DPPH solution

Blank = the absorbance of sample and EtOH solution

Then, IC_{50} (50 % oxidative inhibitory concentration) values were calculated by linear regressive excel program.

Antimicrobial activity screening

The antimicrobial activity of ethanol and watery extracts of *B. purpurea* leaf was investigated by agar well diffusion method at Development Centre of Pharmaceutical and Food Technology (DCPFT). The microorganisms used in this study included five bacterial strains such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *E. coli* and one fungal species, *Candida albicans*.

Acute toxicity study

The acute toxicity activity of ethanol extract of *B. purpurea* leaf was done according to the Organization for Economic Cooperation and Development (OECD) guide line 423 (2001). According to the test description, total number of 18 adult female albino mice, weighing (25-30 g) were selected and divided into three groups (six in each group). Each group was treated with ethanol extract in different concentrations (300, 2000, 5000 mg/kg body weight) by using a stomach tube. After administration of the test agent orally, the sign of toxicity or lethality was observed on the test animals. At the end of the test (i.e., 14 days), surviving animals were weighed.

Results and Discussion

Phytochemicals Present in Leaf of *B. purpurea*

In order to find out the types of phytochemical constituents present in *B. purpurea* leaf, phytochemical investigation was carried out by chemical methods. Phytochemical investigation of plant material was done by based on the results of colour changes or precipitation, which indicates that the presence of classes of organic constituents containing in it. According to these results, α-amino acids, flavonoids, glycosides, phenolic compounds, saponins, tannins, steroids and terpenoids were present in sample. However, alkaloids, carbohydrates, cyanogenic glycosides, reducing sugars and starch were not detected.

Some Elements Present in Leaf of *B. purpurea*

The relative abundance of elements present in leaf of *B. purpurea* was determined by EDXRF spectrometer. The EDXRF spectrum is described in Figure 2 and the relative abundance of some elements present in *B. purpurea* leaf is shown in Table 1. It was observed that Ca was found to be the principal element and K, Fe, Sr, Mn, Zn, Cu, Rb and Br were present as trace elements in the sample. Ca is the mineral that human requires in the greatest amounts. It is an essential for the development, growth and maintenance of bone. It helps regulate muscle contraction. K is an important for muscle function, including relaxing the walls of the blood vessels. This lowers blood pressure and protects against muscle cramping. Fe is a mineral vital to the proper function of hemoglobin, a protein needed to transport oxygen in the blood. Therefore, *B. purpurea* leaf contained many necessary elements and it could be a good supplement for some nutrients.

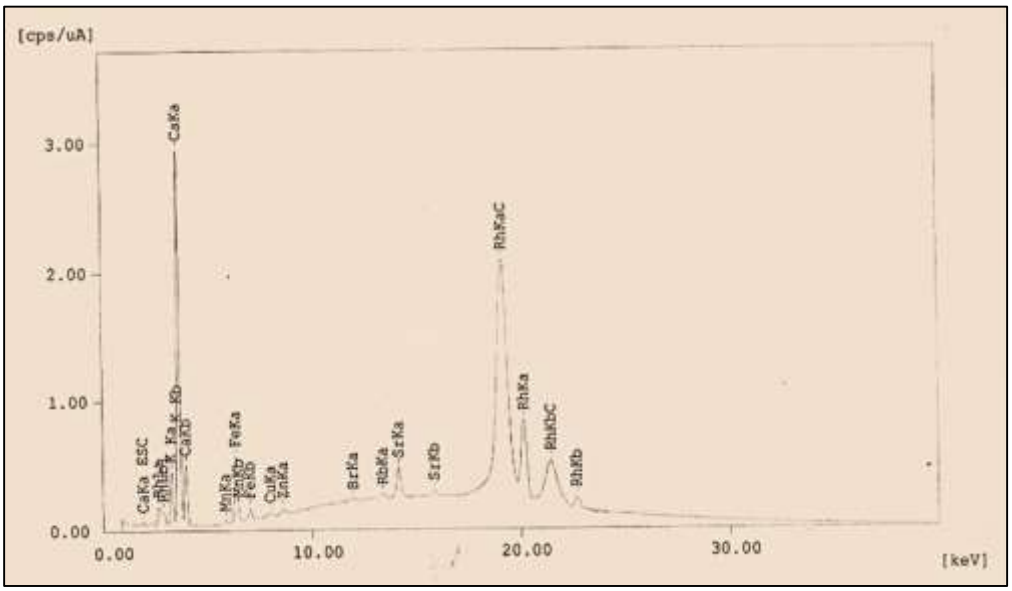

Figure 2 EDXRF spectrum of leaf of *B. purpurea*

Table 1 Some Elements Present in Leaf of *B. purpurea* (EDXRF)

No.	Elements	Relative Abundance (%)
1	Ca	0.818
2	K	0.104
3	Fe	0.059
4	Sr	0.007
5	Mn	0.004
6	Zn	0.003
7	Cu	0.002
8	Rb	0.001
9	Br	0.001

Soluble Matter Contents of Leaf of *B. purpurea*

In this research work, the soluble matter contents of leaf of *B. purpurea* were determined by solvent extraction method. Ethanol and watery crude extracts were prepared and the yield percent of crude extract was derived from the weight of dried and ground plant material. According to the results, it was observed that the yield of ethanol extract (15.93 %) was higher than that of watery extract (12.58 %). It can be concluded that the amount of active constituents contained in ethanol extract was higher than watery extract.

Total Phenolic Content of Leaf of *B. purpurea*

Phenolic compounds are very important plant constituents because of their scavenging ability due to their hydroxyl groups. They may contribute directly to antioxidative action. The total phenolic contents in ethanol and watery extracts of leaf of *B. purpurea* were determined by using Folin-Ciocalteu colourimetric method. Gallic acid was used as a standard compound. The absorbance values obtained at different concentrations of gallic acid were used for the construction of calibration curve as described in Figure 3. The total phenolic content of crude extracts of *B. purpurea* leaf is shown in Table 2. This method is based on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybedic / phosphotungstic acid complexes to form blue coloured complexes that are determined spectrophotometrically at 765 nm. Total phenolic content of each crude extract was calculated from the regression equation of calibration curve ($y = 0.008$ x, $R^2 = 0.999$) and expressed as microgram gallic acid equivalent per milligram of crude extract (µg GAE/mg of extract). In this study, ethanol extract (32.58 µg GAE/mg of extract) showed higher total phenolic content than watery extract (21.73 µg GAE/mg of extract). This means that ethanol extract of *B. purpurea* leaf is a source phenolic compound which may be a good source of antioxidant for the food system.

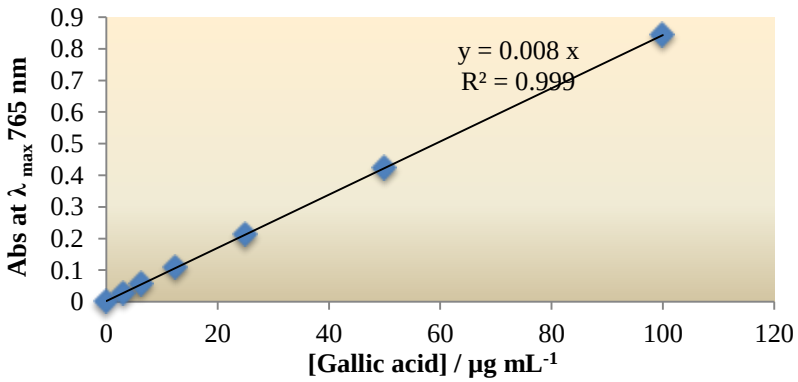

Figure 3 Standard calibration curve of gallic acid

Table 2 Total Phenolic Content of Crude Extracts of *B. purpurea* Leaf

Extracts	Total Phenolic Content (µg GAE/mg of extract)
ethanol	32.58
watery	21.73

Antioxidant Activity of Leaf of *B. purpurea*

The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen or electron donating abilities. DPPH is a stable free radical. In its radical form, DPPH has been disappeared on reduction by an antioxidant compound or a radical species to become a stable diamagnetic molecule resulting the colour changes from violet to yellow, which could be taken as an indication of the hydrogen donating ability of the tested sample.

The antioxidant activity of ethanol and watery extracts of leaf of *B. purpurea* was investigated by DPPH assay. In this study, six different concentrations (50, 25, 12.5, 6.25, 3.125 and 1.5625 µg/mL) of each crude extract were prepared by serial dilution method. Ascorbic acid was used as standard to be compared with the sample and ethanol without sample was employed as control. After mixing with the DPPH solution, the absorbance of each solution was measured at 517 nm using UV-visible spectrophotometer. On the basic of absorbance values, % inhibition of each sample in different concentrations was calculated and IC_{50} value was determined by linear regressive excel program. The results of % inhibition and IC_{50} values were shown in Table 3. IC_{50} value of standard ascorbic acid was 1.97 µg/mL. According to the results, it was observed that ethanol extract (IC_{50} = 5.75 µg/mL) of leaf of *B. purpurea* contained higher antioxidant activity than watery extract (IC_{50} = 8.51 µg/mL), due to the lower value of IC_{50}. The ethanol extract contains a high quantity of bioactive compounds able to capture free radicals like DPPH. From these findings, it can be concluded that total phenolic content was correlated with radical scavenging activity. The ethanol extract containing the higher total phenolic content showed significant free radical scavenging activity in this study. Therefore, *B. purpurea* leaf could be employed as an additive in the food industry providing good production against oxidative damage.

Table 3 Percent Inhibition and IC_{50} Values of Crude Extracts of *B. purpurea* Leaf and Standard Ascorbic acid

Sample	% inhibition in various concentrations (µg/mL)						IC_{50}
	1.5625	3.125	6.25	12.5	25	50	(µg/mL)
ethanol extract	23.76	32.18	53.42	71.56	89.34	92.42	5.75
watery extract	19.02	24.87	40.93	65.98	82.76	87.43	8.51
ascorbic acid	44.56	65.32	74.42	85.96	93.21	97.12	1.97

Antimicrobial Activity of Leaf of *B. purpurea*

The antimicrobial activity of ethanol and watery extracts of *B. purpurea* leaf was investigated by agar well diffusion method on *B. subtilis, S. aureus, P. aeruginosa, B. pumilus, C. albicans* and *E. coli* as shown in Figure 4. The zone of inhibition was taken as a measure of antimicrobial activity. The larger the inhibition zone diameter, the higher the antimicrobial activity. All tested microorganisms in this study were found to be sensitive to both of the ethanol and watery

extracts of *B. purpurea* leaf. *B. subtilis* showed the highest susceptibility to both extracts with the clear zone of inhibition ranging from 18-20 mm while the rest tested microorganisms were sensitive in the inhibition zone ranging from 14-18 mm as shown in Table 4. The results indicated that the leaf of *B. purpurea* has antimicrobial activity and may be applied in local therapies in the treatment of diseases caused by the microorganisms tested.

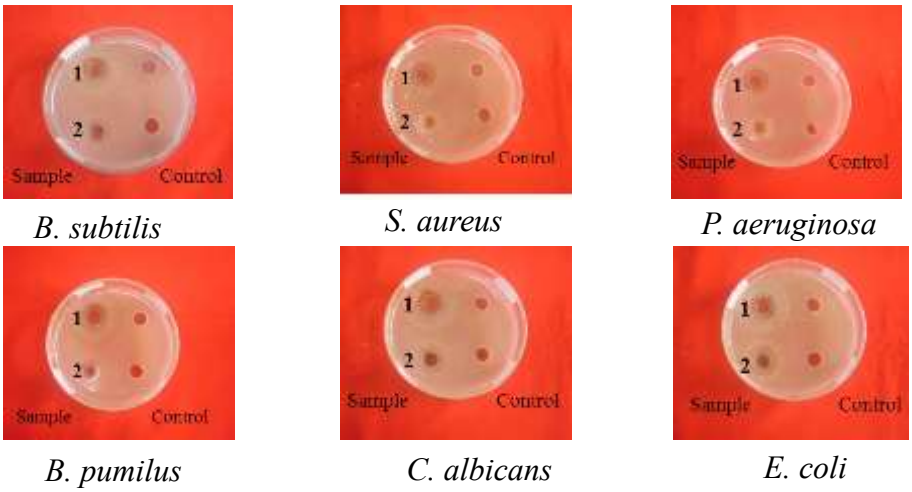

Figure 4 Antimicrobial activity of crude extracts of *B. purpurea* leaf on six microorganisms

(1) = ethanol extract

(2) = watery extract

Table 4 Inhibition Zone Diameters of Crude Extracts of *B. purpurea* Leaf

Extracts	Microorganisms					
	B. subtilis	***S. aureus***	***P. aeruginosa***	***B. pumilus***	***C. albicans***	***E. coli***
ethanol	20 mm (+++)	18 mm (++)	18 mm (++)	18 mm (++)	18 mm (++)	17 mm (++)
watery	18 mm (++)	16 mm (++)	17 mm (++)	14 mm (+)	16 mm (++)	15 mm (++)

Agar well diameter 10 mm-14 mm (+)
15 mm-19 mm (++)
20 mm above (+++)

Acute Toxicity Study of Leaf of *B. purpurea*

The acute toxicity study of ethanol extract was done according to the OECD 423 guide line. In this study, no toxic sign and lethality were found during the observation period of 14 days with the dose of 300 mg/kg, 2000 mg/kg and also at the maximum dose of 5000 mg/kg. Therefore, the LD_{50} value was expected to be more than 5000 mg/kg and ethanol extract leaf of *B. purpurea* is assumed to be safe. The results of acute toxicity activity of ethanol extract of leaf *B. purpurea* were shown in Table 5.

Table 5 Acute Toxicity Activity of Ethanol Extract of *B. purpurea* Leaf

Extract	Dose (mg/kg)	Number of mice tested	Observed period (days)	Death/tested
Ethanol	300	6	14	0/6
	2000	6	14	0/6
	5000	6	14	0/6

Conclusion

This study showed that there is a relationship between, the total phenolic content of ethanol and watery extracts of leaf of *B. purpurea* with antioxidant activity indicating that *B. purpurea* leaf has potent antioxidant, antimicrobial and no acute toxicity activities and provides scientific evidence for its use in traditional medicine. Though the chemical nature were not identified yet, however, the leaf of *B. purpurea* may be used for the treatment of diseases caused by oxidative stress and infected by the microorganisms.

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