Study on Preliminary Phytochemical Constituents, Nutrient Minerals and Antimicrobial Screening of *Buteamonosperma*(Lam.) Taub. Flower Collected from Hinthada University Campus

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Abstract

Buteamonosperma (Lam.) Taub. Flower, used in traditional medicine, was chosen for the present study. The aim of the study is to examine phytochemicals, nutrient minerals and antimicrobial screening of *Buteamonosperma* flower. At first, preliminary phytochemical tests have revealed the absence of cyanogenic glycosides in the sample according to test tube method. As the nutrient minerals, flower sample relatively contained the highest content of K, Ca and P whereas minor component of S, Fe, Cu, Mn, Ti, Zn, Rb and Ni according to Energy dispersive X-ray fluorescence (EDXRF) spectrum. By using solvent extraction method, four crude extracts of *Buteamonosperma* flower were prepared by petroleum ether, ethyl acetate, 96% ethanol and water solvent. The antimicrobial activity of four crude extracts was investigated against eight tested microorganisms by agar well diffusion method. It was found that all tested samples except petroleum ether and water extracts showed antimicrobial activity against all tested microorganisms with the range of zone diameter between 12~24 mm. The results showed that it could be used as the local health remedy to the local indigenous communities of our country.

Keywords*:* *Buteamonosperma* flower, phytochemicals, nutrient minerals, antimicrobial activity

INTRODUCTION

In Myanmar, gastrointestinal (GI) problem is one of the major health problems especially in Ayeyarwady Region which local people face dysentery and diarrhea due to the consequences of the flood. In fact, a localized problem was related to public health.Therefore, the present research was designated to explore the promising Myanmar medicinal plant in the use of traditional drug for food poisoning, typhoid and diarrhea related to GI problems. Moreover, it was studied to know how much nutrient elements and medicinal values contained in the selected sample. The plant of *Buteamonosperma* and its flower shown in Figure 1 (a) and (b), family Fabaceae (Meena and Ramaswamy, 2014) Myanmar name: Pauk and common name: flame of the forest is well known for medicinal purposes (Kress, 2003) besides its lovely flower (Burlia and Stassen, 2007). In front of Hinthada University, the red flowers of *Buteamonosperma* are very beautiful in summer. In addition, Pauk flowers belong to medicinal properties especially used in diarrhea, skin ulcers, piles, eye diseases, impotency and menstrual cramps (Somani, *et al*., 2006). The plant is a tropical tree, 12m in height (Muthuswamy and Senthamarai, 2014) and beautiful with orange-red flowers (Sindhia and Bairwa, 2012). It is widely distributed in Myanmar. This research can contribute to local indigenous medicine and may solve some problems related to GI and skin diseases. In the study, the sample collection, examination of phytochemicals, investigation of nutrient elements, preparation of various crude extracts and screening of antimicrobial activity from the flower sample have been carried out.

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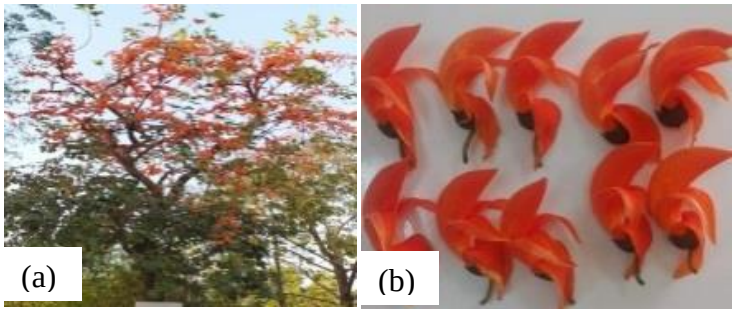


Fig 1. (a) *B. monosperma* (Pauk tree**)** (b) *B. monosperma*(flower)

MATERIALS AND METHODS

Plant Materials

The sample of *Buteamonosperma* flower was collected from Hinthada University campus, Ayeyarwady Region in March, 2020. The plant was identified by plant taxonomists at Department of Botany, Hinthada University. The flower sample was cleaned and dried at room temperature for two weeks. Then, the dried sample was powdered and stored in air- tight container to avoid moisture changes and other contamination.

Preliminary Phytochemical Tests of *B. monosperma* Flowerby Using Test Tube Method

The dried powder sample of *B. monosperma* flower was used to carry out Preliminary phytochemical investigation by Test Tube Method.

Determination of Nutrient Minerals from *B. Monosperma* Flower by EDXRF Spectrometry

The dried powder sample of *B. monosperma* flower was analyzed by Energy dispersive X-ray fluorescence spectrometer (Shimadzu's EDX-7000/8000) at Monywa University. The dried powder sample was fabricated into pellet by using pellet making machine. It can analyze the elements (minerals) from sodium to uranium. The individual elements comprising in the sample re-emit their own characteristic X-rays. The X-rays are detected by using semiconductor detector [Si (Li)] that permits multi-element, simultaneous analysis.

Preparation of Crude Extracts of *B.monosperma* Flower by Solvent Extraction Method

The powdered sample of *B. monosperma* flower (50 g) was extracted with (500 mL) petroleum ether (PE), ethyl acetate (EtOAc) and 96 % ethanol (EtOH) in separate conical flasks, respectively for at least three weeks and then filtered. Water extract of flower sample was prepared by boiling 50 g of sample with 500 ml of distilled water for 6 h and filtered. The filtrates were evaporated by using rotary evaporator and desiccated and also weighed. Extractive values were described in terms of % in weight by weight on the powdered materials. Solvent was removed as before until a constant weight of extracts was obtained. The four crude extracts from *B. monosperma* flower were applied to investigate antimicrobial activity.

Antimicrobial Activity of Crude Extracts of *B. monosperma* Flower by Agar Well Diffusion Method

For the examination of *in vitro* antimicrobial activity, agar well diffusion method was used because of its simplicity, speed of performance, economy and reproducibility (Ando, 2014).The antimicrobial activity of four crude extracts: PE, EtOAc, 95 % EtOH and H_2O extracts were determined against eight microorganisms such as *Escherichia coli* AHU5436, *Bacillus subtilis* IFO90571, *Bacillus pumilus* IFO12092, *Candida albicans* NITE09542,

Pseudomonas fluorescens IFO94307, *Staphylococcus aureus* AHU8465, *Agrobacterium tumefaciens* NITE09678 and *Malassezia furfur* AUV0255by employing agar well diffusion method at Department of Chemistry, Hinthada University. These eight tested microorganisms were obtained from the sources of NITE and Kyowa Hakko Co. Ltd., Japan and University of Yangon (UY).These microorganisms were cultured at Biotechnology and Development Center of Pathein University. The test procedure was as follows: the test samples (1 g each) were dissolved in 1 mL of appropriate soluble solvent, and introduced into sterile petridishes for testing eight cultured microbial strains. The test organisms were incubated in test broth medium containing glucose (0.5 g), yeast (0.3 g), peptone (0.2 g) and distilled water (100 mL) at 27°C for 24 h. Assay medium containing glucose (50.0 g), peptone (30.0 g), yeast (30.0 g), agar (14.0 g) and distilled water (1000 mL) were placed in a beaker and the contents were heated for 10 min. The assay medium was put into sterilized conical flask and plugged with cotton wool and then autoclaved at 121 °C for 15 min. After cooling down to 40 °C, 0.1 mL of suspended strain was inoculated to the assay medium with the help of a sterilized disposable pipette near the burner. About 20 mL of medium was poured into the sterilized petri-dishes and allowed to set the medium. Once solidified, the dishes were cooled for 2 h at room temperature. After solidification, the agar well was made with 8 mm sterile cork borer from each agar. After inoculums had been dried for 5 min, the wells were filled with test sample (0.2 mL extracts) to be tested. After 24-48 h incubation at 27 °C, the zones of inhibition diameter including 8 mm well were measured with digital calipers in millimeter. Clear zones (inhibitory zones) surrounding the agar well were found to be indicated that it would be the presence of bioactive metabolites which inhibit the growth of test organisms. The incubated petri-dish without test sample was taken as control and antibiotics; chloramphenicol was used as standard for this study.

RESULTS AND DISCUSSION

Preliminary Phytochemical Tests of *B. monosperma* Flower

Phytochemical screening serves as an initial step to recover new sources of phytochemical and bioactive constituents. For the reasons mentioned above, the preliminary phytochemical Tests of *B. monosperma* Flower was carried out and it was found that the flower sample consists of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugar, saponins, starch, steroids, tannins and terpenoids. However, cyanogenic glycosides (plant toxin), were not found in it. Alkaloids possess the properties of antitumor, antiviral, antihypertensive, antidepressant, antimicrobial and anti-inflammatory activities. Flavonoids are used in antibacterial, anti-inflammatory, anti-allergies, anti-mutagenic, antiviral and antithrombotic activity. Glycosides are used as antibiotic, anticancer, antidiabetic, purgative, treatment of congestive heart failure and cardiac arrhythmia. Phenols are strong antioxidants and they are very useful in prevention and management of chronic diseases (cancer and cardio vascular diseases). Saponins are used for antiviral, antiviral, anti-inflammatory, anti-helminthic, anticancer and anti-cytotoxic activity. Steroids are used as anti-inflammatory, antitumor, anti-allergies, anti-asthma, anti-eczema, anti-arthritis. Tannins are also for antiviral, antibacterial and antitumor properties. The uses of terpenoids include antimicrobial, antifungal, antiviral, anti-hyperglycemic, anti-inflammatory, antioxidant and anti-parasitic (Brahmkshatriya and Brahmkshatriya, 2013). From this study, it could be denoted that the selected sample, *B. monosperma* flower may be a good source of herbal medicine. These results are shown in Figure 2 and Table 1.

Table (1) Results of Preliminary Phytochemical Examination of *B. monosperma* Flower

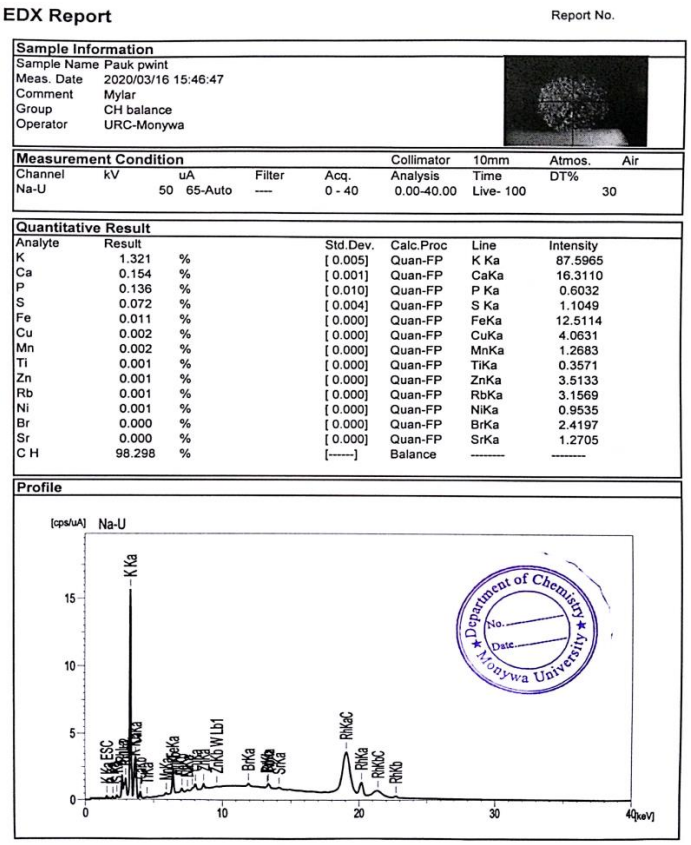
No.	Test	Extracts	Reagents used	Observation	Remark
1	Alkaloids	1 % HCl	Mayer's reagent	Creamy ppt	+
			Dragendorff's reagent	Orange ppt	+
			Wagner's reagent	Reddish brown ppt	+
			Sodiumpicrate solution	Yellow ppt	+
2	α-amino acids	H_2O	Ninhydrin reagent	Purple colour	+
3	Carbohydrate	H_2O	10 % α-naphthol & conc: H_2SO_4	Red ring	+
4	Cyanogenic glycosides	H_2O	conc: H_2SO_4 & sodium picrate solution	No brick red	-
5	Flavonoids	EtOH	Dil. NaOH & Dil. HCl	Yellow colour	+
6	Glycosides	H_2O	10 % lead acetate	White ppt	+
7	Organic acids	H_2O	Bromocresol green	Blue colour	+
8	Phenolic compounds	H_2O	1 % K_3FeCl_6& 5 %$FeCl_3$ solution	Deep blue colour	+
9	Reducing sugars	H_2O	Benedict's solution	Green colour	+
10	Saponins	H_2O	Distilled water	Marked Frothing	+
11	Starch	H_2O	Iodine solution	Blue colour	+
12	Steroids	PE	Acetic anhydride & conc: H_2SO_4	Green colour	+
13	Tannins	EtOH	1 % Gelatin	White ppt	+
14	Terpenoids	EtOH	Acetic anhydride & Conc. H_2SO_4	Red colour	+

(+) = presence (-) = absence

Figure (2) Preliminary phytochemical investigation of *B. monosperma* Flower

EDXRF Analysis of *B. monosperma* Flower

Under vacuum condition, an EDXRF spectrum of *B. monosperma* flower sample is shown in Figure (3). In *B. monosperma*flower, three kinds of minerals such as K, Ca and P were found to be relatively high whereas S, Fe, Cu, Mn, Ti, Zn, Rb and Ni were minor components in the sample. Among them, potassium peak was also the most prominent and so it showed potassium was the highest content of the flower sample. According to the literature survey, potassium deficiency can cause nervous irritability, mental disorientation, low blood sugar, insomnia and coma. Calcium plays a very important role in bones, teeth, muscles system and heart functions. Phosphorus need along with calcium to build strong healthy bone (WHO, 2005). In fact, potassium (K), calcium (Ca), phosphorous (P) and sulphur (S) are important macro minerals which are needed in a large amount for human body. Iron (Fe), copper (Cu), manganese (Mn), titanium (Ti), zinc (Zn), rubidium (Rb) and nickel (Ni) are micro minerals required in a very small amount for human body. From this study, the macro minerals were found to be the major components in selected flower sample. The results of the current study are described in comparison with the other study (Table 2). In this study, the qualitative variations of same species between the current study and the other study may attribute to difference in geographical location, climatic conditions, time of harvest, habitat of plant samples, part used, extraction method, types of solvent and other environmental factors. From the results, it could be deduced that *B. monosperma* flower may support human health. In addition, the investigation of elemental contents can determine physicochemical properties of the flower sample, as well as retard the growth of bacteria.



EDX Report

Report No.

Sample Information

Sample Name	Pauk pwint
Meas. Date	2020/03/16 15:46:47
Comment	Mylar
Group	CH balance
Operator	URC-Monywa

Measurement Condition

Channel	kV	uA	Filter	Acq.	Collimator	10mm	Atmos.	Air
Na-U	50	65-Auto	----	0 - 40	Analysis	0.00-40.00	Time	Live- 100
					DT%			30

Quantitative Result

Analyte	Result		Std.Dev.	Calc.Proc	Line	Intensity
K	1.321	%	[0.005]	Quan-FP	K Ka	87.5965
Ca	0.154	%	[0.001]	Quan-FP	CaKa	16.3110
P	0.136	%	[0.010]	Quan-FP	P Ka	0.6032
S	0.072	%	[0.004]	Quan-FP	S Ka	1.1049
Fe	0.011	%	[0.000]	Quan-FP	FeKa	12.5114
Cu	0.002	%	[0.000]	Quan-FP	CuKa	4.0631
Mn	0.002	%	[0.000]	Quan-FP	MnKa	1.2683
Ti	0.001	%	[0.000]	Quan-FP	TiKa	0.3571
Zn	0.001	%	[0.000]	Quan-FP	ZnKa	3.5133
Rb	0.001	%	[0.000]	Quan-FP	RbKa	3.1569
Ni	0.001	%	[0.000]	Quan-FP	NiKa	0.9535
Br	0.000	%	[0.000]	Quan-FP	BrKa	2.4197
Sr	0.000	%	[0.000]	Quan-FP	SrKa	1.2705
C H	98.298	%	[------]	Balance	--------	--------

Profile

Figure 3. EDXRF spectrum of *B. monosperma* Flower

Table (2) Minerals Contents in *B. Monosperma* Flower of Current Study and Other Study

No.	Element	Relative abundance (%) of present study	Relative abundance (%)* of other Study
1.	K	1.321	1.98
2.	Ca	0.154	1.02
3.	P	0.136	0.27
4.	S	0.072	0.1
5.	Fe	0.011	0.013
6.	Cu	0.002	0.002
7.	Mn	0.002	0.002
8.	Ti	0.001	ND
9.	Zn	0.001	0.007
10.	Rb	0.001	ND
11.	Ni	0.001	ND
12.	C/H	98.298	96.606

*Shruti, *et al*., (2014), ND = not detected

Assessment of Four Crude Extracts from *B. monosperma* Flower by Various Solvents

The dried powder of *B. monosperma* flower collected from Hinthada University campus was extracted by various solvents and the yield % of petroleum ether extract (12.13 %), ethyl acetate extract (18.62 %), 96 % ethanol extract (23.30 %) and water extract (25.20 %) were obtained. It was assumed that polyphenolic and flavonoid compounds may involve in higher yielded 96 % ethanol and water extracts. Therefore, it could be denoted that *B. monosperma* flower possesses medicinal properties.

Investigation of Antimicrobial Activity of Four Crude Extracts from *B. monosperma* Flower by using Agar Well Diffusion Method

In the present study, the antimicrobial activity was performed with particular reference to pathogenic microorganism potential to cause GI tract infection, diarrhea, septicemia, crown gall disease, food poisoning, dandruff, seborrheic dermatitis and abscess in skin, mouth, nose etc. In this work, antimicrobial activity of four crude extracts from flower sample was investigated on eight species of microorganisms by agar well diffusion method at Department of Chemistry, Hinthada University. Agar well diffusion method is based on the inhibition zone diameter in millimeter (mm) of the well. The larger the zone diameter is the more activity on the tested microorganisms is. In this study, the assay medium in absence of samples with culture microorganisms was utilized as control. The four crude extracts were tested with eight microorganisms such as *Escherichia coli* AHU5436, *Bacillus subtilis* IFO90571, *Bacillus pumilus* IFO12092, *Candida albicans* NITE09542, *Pseudomonas fluorescens* IFO94307, *Staphylococcus aureus* AHU8465, *Agrobacterium tumefaciens* NITE09678 and *Malassezia furfur* AUV0255. The measurable inhibition zone diameter of four crude extracts showed the degree of antimicrobial activity (Figure 4 and Table 3). From the results, it was observed that ethyl acetate and ethanol extracts of flower sample exhibited against all tested microorganisms with the inhibition zone diameters between 12~13 mm and 18~24 mm respectively whereas petroleum ether and water extracts didn't show the activity. Out of these extracts, ethanol extract showed the most potent to the tested microorganisms. Nevertheless, ethyl acetate extract of flower sample revealed the low inhibition to eight microorganisms in comparison with the inhibition zone of standard chloramphenicol (20~29 mm). According to the results, ethanol extract of *B. monosperma* flower may be effectively used as an active remedy for the treatment of their related diseases and fungal infection.

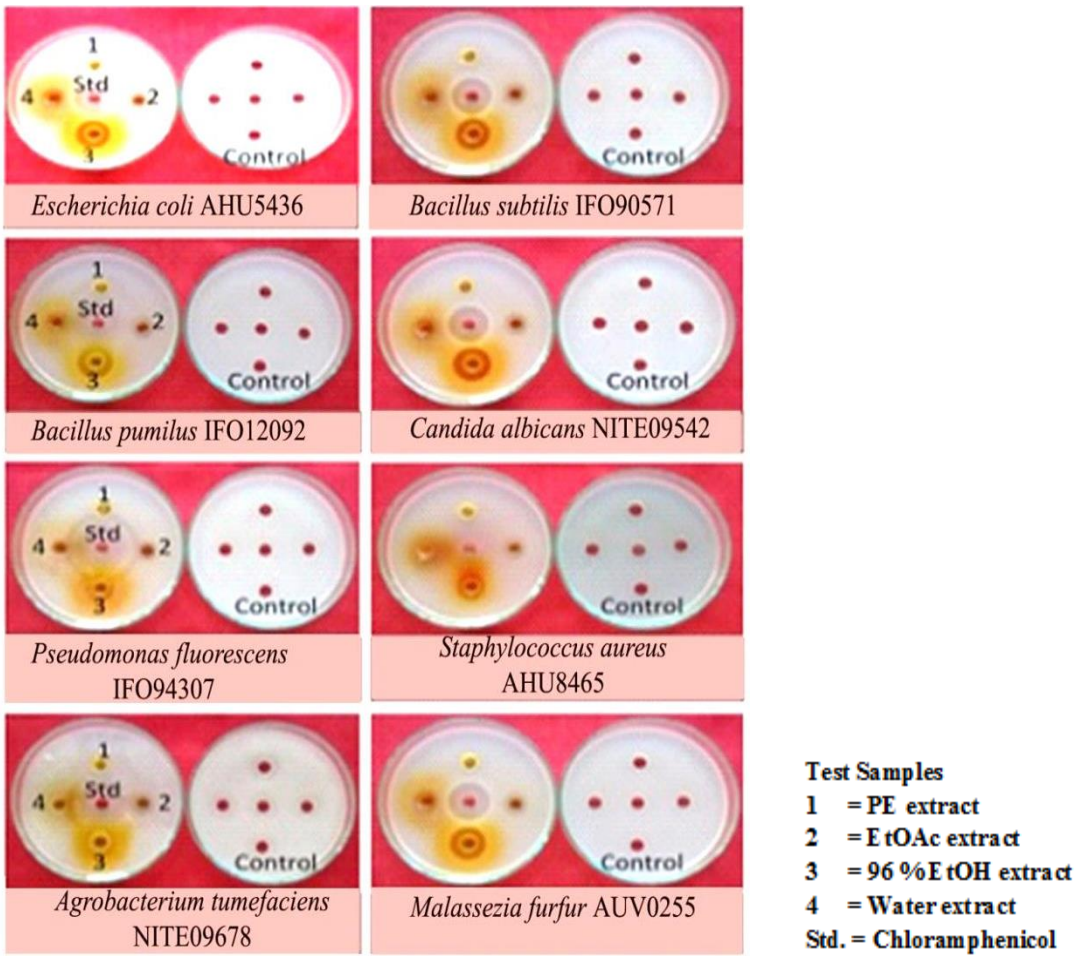


Figure (4) Inhibition zones of four extracts from *B. monosperma* flower against eight Microorganisms

Table (3) Inhibition Zone Diameters of Four Crude Extracts from *B.monosperma* Flower

Microorganisms*	Inhibition Zone Diameters (mm) of Test Samples				
	PE extract	EtOAc extract	96 % EtOH extract	Water extract	Std.
Escherichia coli AHU5436	-	13	19	-	23
Bacillus subtilis IFO90571	-	13	19	-	22
*Bacillus pumilus*IFO12092	-	13	20	-	24
Candida albicans NITE09542	-	12	21	-	22
Pseudomonas fluorescens IFO94307	-	12	24	-	29
Staphylococcus aureus AHU8465	-	12	18	-	20
Agrobacterium tumefaciens NITE09678	-	12	18	-	27
Malassezia furfur AUV0255	-	13	21	-	23

Std. =Chloramphenicol (standard)

Agar well– (8mm)

10 mm-14 mm (+), 15mm-19 mm (++), 20 mm-above (+++)

(-) = no activity, (+) = low activity, (++) = medium activity, (+++) = high activity

Tested microorganisms – From the source of NITE & Kyowa Hakko Co. Ltd., Japan, and University of Yangon (UY) *

CONCLUSION

In the phytochemical analysis, it was concluded that *B. monosperma* (Pauk) flower contained many biological active phytochemicals. In addition, it revealed the absence of plant toxin, cyanogenic glycosides in the flower sample. According to the mineral evaluation, the presence of considerable amount of minerals from Pauk flower could be used as a good supplement of human nutritional requirement for normal growth and adequate protection against diseases arising from malnutrition related to the mineral deficiency. By using solvent extraction method, four crude extracts were prepared from flower sample using petroleum ether, ethyl acetate, 96 % ethanol and water as their solvent polarity. These extracts were used to test antimicrobial activity. Screening of antimicrobial activity of these four crude extracts was also investigated by employing agar well diffusion method against eight microorganisms responsible for GI tract infection, diarrhea, septicemia, crown gall disease, food poisoning, dandruff, seborrheic dermatitis and abscess in skin, mouth, nose etc. It was observed that ethanol extract of flower sample exhibited the most potent inhibition zone diameters between 18~24 mm against all tested microorganisms whereas petroleum ether and water extracts didn't show the activity. Based on the finding of the present study, it is concluded that *B. monosperma* flower possesses medium antimicrobial activity. Since some nutrient elements were also found in *B. monosperma* flower, it could be expected to become a potential food and drug source. It is recommended that further investigations on vitamins, amino acids, isolation of active compounds and efficacies of this plant are needed for novel herbal medicine.

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