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Ethnomedicinal value, phytochemical composition and bioactivity of *Butea monosperma* (Lam.) Taub.

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Abstract

Ethnomedicinal study on *Butea monosperma* (Lam.) Taub. revealed that native people of Netrokona district extensively use flower, bark and leaves of the plant to treat different kinds of diseases. People of the studied area used the plant to treat goiter, diabetes, painful menstruation, body swellings, intestinal worms, urinary stone, leucorrhoea and chronic fever. Application of root powder mixed with honey as an antidote for snake bite was recorded for the first time. Phytochemical screening of the methanolic extracts of flowers, leaves and stem of this plant showed the presence of carbohydrate, flavonoid, glycosides, saponins, terpenoids and steroids. Through qualitative assessment, flower was found to be rich in flavonoids compared to leaf and stem. Leaf extract of *B. monosperma* showed relatively higher cytotoxicity than flower and stem extracts. The highest free radical scavenging activity was observed in flower sample (73.49%) and the lowest in leaf sample (48.17%). The results of the present study may be a proof of a scientific basis for the use of *B. monosperma* in traditional medicine.

Key words: *Butea monosperma*, phytochemical composition, bioactivity, TPC.

INTRODUCTION

Bangladesh is a home to a number of tribes or indigenous communities. Latest ethnographic research suggests that the number of tribes within the country approximates more than 100 instead of the previously estimated about a dozen tribes (Murmu, 2009). Traditional medicine practices and ethnobotanical information play an important role in the scientific research, particularly when the literature and fieldwork data have been properly evaluated. The knowledge of ethnobotanical use of plants often results in the discovery of new biologically active molecules (Gurib-Fakim, 2006). According to WHO, medicinal plants would be the best source to obtain a variety of drugs. Systematic screening of folk medicines and plants may result in the discovery of novel effective compounds (Tomoko *et al.*, 2002).

There are around 5,000 angiosperms distributed among 200 families in Bangladesh and approximately, 500 of these are being used in the traditional medicines for the treatment of different types of diseases (Rashid *et al*., 2014). Among them, *Butea monosperma* (Family: Fabaceae), popularly known as 'palas' in Bengali, has been found to display a wide variety of biological activities. It is a moderate sized deciduous tree and widely distributed throughout Bangladesh, Myanmar, Ceylon and India. The plant is traditionally reported to possess astringent, bitter, alterative, aphrodisiac, anthelmintic, antimicrobial,

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anthelmintic, antidiabetic, diuretic, analgesic, antitumor, astringent and anti-asthmatic properties (Neelam *et al*., 2015; Rana & Avijit, 2012; Shrestha & Dhillion, 2003).

Netrokona district is located in between 24°34' and 25°12' north latitudes and in between 90°00' and 91°07' east longitudes. It is bounded by the Meghalaya state of India on the north, Kishoreganj district on the south, Sunamganj district on the east and Mymensingh district on the west. Garo, Hajong, Hodi and Banai are the minor indigenous communities among the population of Netrokona district (BBS, 2007). Being a peripheral district most of the indigenous people are on the verge of disappearance because of decline in population, loss in tribal habitat, or because of merging with the mainstream Bengali-speaking population. Madan and Kendua Upozilla of Netrokona district is well known for its diverse inhabitants and cultural practice. Since ethnomedicinal surveys of various tribes and folk medicinal practitioners are still at an early stage in Bangladesh, the primary objective of the present study was to document the hitherto unreported traditional medicinal practices of *B. monosperma* in the villages of Madan and Kendua Upozilla of Netrokona district. Till to date no document came up with the information on ethnomedicinal use of *B. monosperma* in Netrokona district of Bangladesh. Thus, the main objectives of the present research aims to prepare a comprehensive documentation of indigenous knowledge on the utilization of *B. monosperma* by local inhabitants and Garo and Hazong ethnic communities, followed by determination of phytochemical composition, cytotoxicity and antioxidant potential of different plant parts.

MATERIALS AND METHODS

Ethnobotanical study and survey: Extensive field trips were conducted for ethnobotanical survey using a semi-structured questionnaire prepared following Martin (2008). Ten kavirajes of two upozilla (Madan and Kendua) of Netrokona District with the age ranging from 50 to 60 years were interviewed individually and they pointed out the use of *B. monosperma* to treat different ailments.

Collection and Identification: Samples of root, stem, leaf, flower and seed of *B. monosperma* plant were collected separately from Netrokona district and deposited in Plant Systematics and Biodiversity laboratory of Jahangirnagar University for preliminary identification. Finally the identification of the plant specimens were verified and authenticated by the Bangladesh National Herbarium (DACB), Mirpur, Dhaka, Bangladesh. The voucher specimen (accession no- DACB-39193) has been deposited in DACB for further reference.

Preparation of crude extracts: The collected plant parts were separately sun-dried followed by drying in a hot air oven (Gallenkamp) at reduced temperature (<50ºC). About 200 g powder of each plant parts was digested with 1000 ml of methanol for three days accompanying with occasional shaking and stirring. The whole mixtures was filtrated by a piece of clean, white cotton material. The extract was concentrated at 45ºC under reduced pressure using a rotary evaporator.

Phytochemical screening: The crude methanolic extracts of flower, leaves and stem were subjected to different qualitative tests to find out the presence of chemical

constituents using standard procedure (Evans, 1989; Sofowara, 1993; Ghani, 1998 and Dev, 2002). Molisch's and Fehling's reagents were used to investigate the presence of carbohydrates and reducing sugar, respectively. Hagger's reagent, Wagner's reagent, Mayer's reagent and Dragendroff's reagents were used to test the presence of alkaloids while $FeCl_3$ test and Keller Killiani's test (Khandelwal, 2008) were carried out for glycosides and cardenolides, respectively. Borntrager's test (Houghton & Rahman, 1998) was conducted to know the presence of anthraquinone glycosides; Lead acetate, Alkali, $FeCl_3$ and Conc. H_2SO_4 were used for the detection of flavonoids. $FeCl_3$, ammonia and lead acetate were used to test the presence of phenolic compounds. Concentrated H_2SO_4 was used to detect terpenoids whereas acetic anhydride was used to check the presence of triterpene. The presence of phytosterols/steroids was indicated by the Salkowski's test (Kokate *et al*., 2008) while the presence of saponins was confirmed by foam test (Kokate *et al*., 2008).

Determination of antioxidant activity: The antioxidant activities of the extracts were measured on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical following the method described by Blois (1958) and Aoshima *et al*. (2004). 150 µl DPPH solution was added to 3 ml methanol and absorbance was taken immediately at 517 nm for control reading. 50 µl of various concentrations of different fractions as well as standard compound (ascorbic acid) were taken and the volume was made uniformly to 150 µl using methanol. Each of the samples was then further diluted with methanol up to 3ml and to each 150 ml DPPH was added. Finally, absorbance at 517 nm was determined after 30 min. and the percent inhibition activity was calculated as

$$\text{DPPH free radical scavenging activity } (\%) = \frac{\text{control absorption - corrected sample absorption}}{\text{control absorption}} \times 100$$

Brine shrimp lethality bioassay: The lethality of a test sample in a simple zoological organism such as the shrimp (*Artemia salina*) has been utilized by Meyer *et al*. (1982) through the Brine Shrimp Cytotoxicity Test (BSCT). It has been well utilized to screen and fractionation of physiologically active plant extracts as well. This bioassay is indicative of cytotoxicity and a wide range of pharmacological activity of natural products. Brine shrimps (*Artemia salina*) lethality bioassay was followed by Meyer *et al*. (1982).

RESULTS AND DISCUSSION

Folk medicine is a traditional form of medicinal practice in Bangladesh, which is practiced by practitioners existing both among the mainstream Bengali-speaking population as well as among the various indigenous communities of Bangladesh. The mainstay of the folk medicinal formulations consist of medicinal plants, which are used directly or in the form of decoctions, juice, pastes and are administered either orally or topically, depending upon the ailments treated. The findings from the ethnomedicinal survey among the local inhabitants including a few ethnic communities (Garo and

Hazong) of Madan and Kendua Upozilla of Netrokona district have been presented in Table 1.

Table 1. Ethnomedicinal information on *Butea monosperma* obtained from Madan and Kendua Upozilla of Netrokona district.

Netrokona District	Village	Name of practitioner	Age (years)	Ailment	Parts used	Process of use
Madan Upozilla	Porashkhila	Mahindro	65	Stomach disorder, cough and cold	Petiole	8-10 leaf petiole is chewed and juice is taken in empty stomach.
	Gobindrosri	Fahima Akter	53	Goiter	Stem	Necessary amount of tender stem is smashed with lime stone and the paste is applied on goiter developed area.
	Gonganagar	Nasrin Akter	56	Snake bite	Root	Two teaspoonful of root powder and a teaspoon of honey mixed with water and drink as an antidote for snake bite.
	Bagjan	Al Amin	50	Diabetes	Leaf	Dried leaf powder, about two spoonful per day over a period of months is drunk mixed with a cup of water to cure diabetes.
	Kapasatia	Prodip Chandro Sutrodhar	58	Painful menstruation	Leaf and Flower	Fresh leaf extract about three to four teaspoon mixed with ¼ teaspoon of fresh flower extract, is drunk at night for two to three months to checks irregular bleeding of menstruation.
Kendua Upozilla	Pubati	Rupok	50	Body swellings	Bark	To cure body swelling, paste of stem and bark together is applied on twice a day.
	Dauki	Chinmoy Dhor	55	Intestinal worms	Seeds	Two to three seeds powdered and consumed by children on a remedy against intestinal worms
	Dipara	Arup Kumar	65	Kidney stone	Seeds	A little amount (unspecified) of seeds are crushed in milk and this mixture about two spoons is taken orally.
	Alampur	Kuddus Ali	60	Leucorrhoea	Flower	7-8 fresh flower is soaked in water over night and a cup of this infusion is drunk every morning.
	Sandikona	Majed Ali	62	Chronic fever	Flower	5-6 fresh flowers are crushed in milk and a teaspoon of sugar is added. Three to four spoons drunk per day over a period of a month.

The Kavirajes were explained properly for providing the information that was wanted, the purpose for obtaining this information, and told that the survey results may be disseminated both nationally and internationally as they prefer to keep their knowledge and formulations, inherited from their forefathers, between themselves.

The survey revealed that stem and flower of *B. monosperma* were the most used parts for medicinal purposes in Netrokona district (Fig. 1). The next popular parts of the plant used were leaves and seeds, where each of them scored 17% of the total usage. Petiole and root of the plant showed least usage (8%). Interestingly, the use of *B. monoperma* root in medicinal purpose was not well recorded. In Bangladesh, this may be the first information on the medicinal use of *B. monosperma* root (Table 1). Patil *et al*. (2006) reported on the similar use of this plant from India. However, the findings on the usage of leaf, stem, flower and seeds of *B. monosperma* plant to treat different diseases of human are corroborated with that of Sharma & Deshwal (2011), Kirtikar & Basu (1935) and Kumar & Samanta (2012).

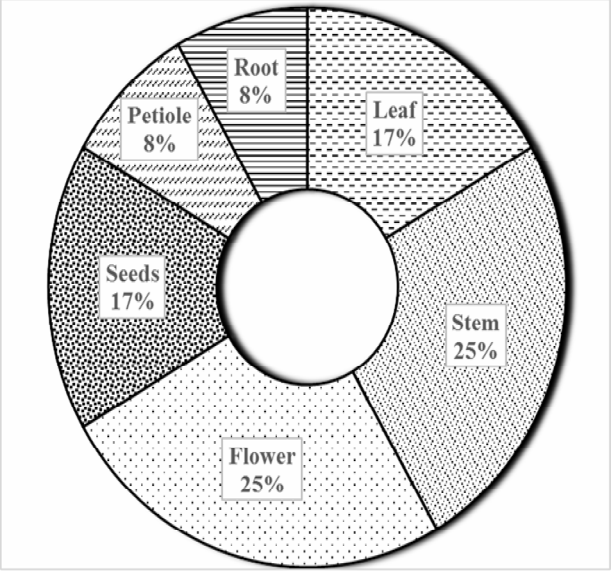

Fig. 1. Comparison among the usage of different parts of *B. monosperma* plant by the Kavirajes of Madan and Kendua upozilla of Netrokona district

The results of phytochemical screening of flower, leaves and stem of *B. monosperma* as presented in Table 2 showed the inconsistent presence of major secondary metabolites, viz. carbohydrates, glycosides, cardiac glycoside, flovonoids, phenolics tannins and terpenoids.

Table 2. Qualitative chemical examination of flower leaves and stem extracts of *B. monosperma*

Sl no.	Phytochemicals	Reagents and tests to detect phytochemicals	Plant samples		
			Flower	Leaves	Stem
01.	Carbohydrates	Molish's reagent	(++)	(++)	(++)
		Fehling reagent	(++)	(++)	(++)
02.	Alkaloids	Hagger's reagent	(-)	(-)	(-)
		Wagner's reagent	(-)	(-)	(-)
		Mayer's reagent	(-)	(+)	(+)
		Draggendrof's reagent	(+)	(+)	(+)
		Tannic acid solution (10%)	(-)	(-)	(-)
03.	Glycosides	$FeCl_3$ glycoside test	(+++)	(++)	(++)
	a. Cardiac glycosides	Keller Killiani's	(+)	(+)	(+)
	b. Anthraquinone glycosides	Borntrager's	(+)	(+)	(-)
04.	Flavonoids	Lead acetate	(+++)	(++)	(++)
		Alkali	(+++)	(++)	(++)
		Conc.H_2SO_4	(++)	(+)	(+)
		$FeCl_3$	(+++)	(++)	(++)
05.	Terpenoids				
	a. Terpene	$CHCl_3$+Conc. H_2SO_4	(++)	(+)	(+)
	b. Triterpene	$CHCl_3$+ Acetic anhydride	(+)	(+)	(+)
06.	Steroids	Salkowski test	(+)	(+)	(+)
07.	Phenols	$FeCl_3$	(+)	(+)	(+)
		Ammonia	(-)	(-)	(-)
		Lead acetate	(+)	(+)	(+)
08.	Saponins	Foam test	(+)	(+)	(+)

NB: +++ = Strong, ++ = Moderate, + = Weak, - = Negative

Crude methanolic flower extract of *B. monosperma* showed strong presence of flavonoids and glycosides, moderate presence of carbohydrates, phenols, tannins and very weak presence of terpenoids and saponins. These results are corroborated with the findings of Ahmed *et al*. (2011). Stem and leaves showed moderate presence of carbohydrates, glycosides, flavonoids, phenols and tannins. Alkaloids, terpenoids and saponins were found to be very feebly present in them (Rajput *et al*., 2011). In the present experiment different plant parts of *B. monosperma* showed varying degree of cytotoxicity. In Brine Shrimp Lethality bioassay, the crude methanolic extract of leaves of *B. monosperma* showed LC_{50} value 427.88 µg/ml after 6 hours followed by the LC_{50} values 691.70 µg/ml for flowers and 809.57 µg/ml for stem extracts. With the progression of hours the cytotoxicity gradually increased. After 12 hours, the LC_{50} values for leaves, flowers and stem were 148.25 µg/ml, 223.03 µg/ml and 463.25 µg/ml respectively. However, after 24 hours the LC_{50} values for flower, leaves and stem were 94.44 µg/ml, 26.06 µg/ml and 113.24 µg/ml respectively (Table 3). Vincristine sulphate was considered as the standard compound to assess the cytotoxicity which showed LC_{50} values 0.069 µg/ml, 0.031 µg/ml

and 0.009 µg/ml after 6 hours, 12 hours and 24 hours respectively. Among the plant parts, leaves extract showed greater cytotoxicity compared to flower and stem extracts. Kumar *et al*. (2009) reported on LC_{50} value 502.41 µg/ml for *Butea frondosa* leaf extracts. Sehrawat & Sultana (2006) reported on the application of leaf extract of *B. monosperma* in treatment against 2-AAF induced hepatic toxicity and hyperproliferation.

Table 3. Cytotoxicity assay of crude methanolic extract of flower, leaves and stem of *B. monosperma*

Duration	Conc. (µg/ml)	Flower		Leaves		Stem		Vincristine sulphate	
		Mortality (%)	LC_{50} (µg/ml)	Mortality (%)	LC_{50} (µg/ml)	Mortality (%)	LC_{50} (µg/ml)	Conc. (µg/ml)	LC_{50} (µg/ml)
6 hours	Control	0	**691.70**	0	**427.88**	0	**809.57**	Control	**0.0699**
	0	0		0		0		0	
	10	0		0		0		0.06	
	20	0		0		0		0.125	
	40	0		0		0		0.25	
	80	0		0		0		0.5	
	160	10		20		10		1	
	200	20		25		15		5	
	400	30		30		20		10	
12 hours	Control	0	**223.03**	0	**148.25**	0	**463.25**	Control	**0.031**
	0	0		0		0		0	
	10	10		10		0		0.06	
	20	15		15		10		0.125	
	40	25		25		15		0.25	
	80	25		25		20		0.5	
	160	45		55		25		1	
	200	65		55		50		5	
	400	90		80		70		10	
24 hours	Control	0	**94.44**	0	**26.06**	0	**113.24**	Control	**0.009**
	0	0		0		0		0	
	10	35		30		15		0.06	
	20	40		45		20		0.125	
	40	45		45		25		0.25	
	80	60		55		50		0.5	
	160	85		80		70		1	
	200	95		90		85		5	
	400	100		100		100		10	

Note: Vincristine sulphate used as a positive control.

Considering the above facts plant samples may be regarded as moderate to poor cytotoxic. Panda *et al*. (2009) isolated a stigmasterol from the bark of *B. monosperma* and evaluated for its thyroid hormone and glucose regulatory efficacy in mice. Rekha (2011) reported that the ethanolic extract of *B. monosperma* showed significant anti-cancer

activity in the tested animal models. However, the present findings may be the first comparative cytotoxicity profile for different parts of *B. monosperma* plant.

The antioxidant activities of flower, leaves and stem extracts were measured as percentage of DPPH free radical scavenging activity and have been presented in Fig. 2. The methanolic extract of the flower of *B. monosperma* showed maximum antioxidant or DPPH free radical scavenging activity (73.49%) followed by scavenging activities of stem (58.58%) and leaves (48.17%) respectively. Ascorbic acid, the reference compound to measure antioxidant potential of the samples, showed 87.07% DPPH free radical scavenging activity. Relatively higher concentrations of the samples showed higher antioxidant activity compared to low and moderate concentrations of the fractions. Present results on antioxidant potential of different parts of *B. monosperma* corroborates with the findings Edwin *et al*., 2009; Lavhale & Mishra, 2007 and Sharma & Garg, 2009.

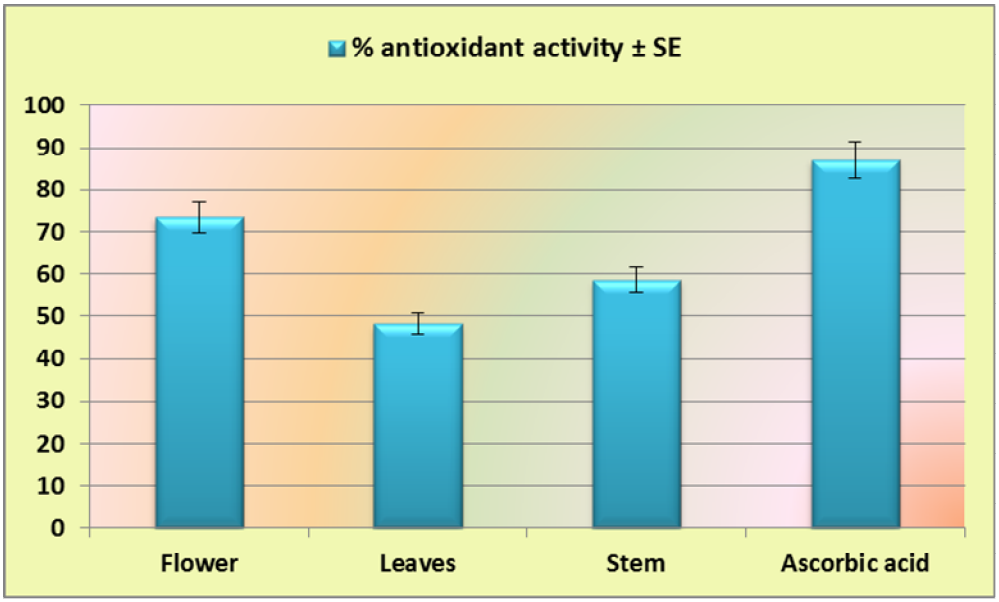

Fig. 2. DPPH free radical scavenging activity of methanolic crude extracts (100 µg/ ml) of flower, leaves and stems of *B. monosperma*

The total phenolic content (TPC) was determined in comparison with standard Gallic acid and the results were expressed in terms of mg Gallic acid equivalent (GAE)/ 100 g dry sample. Among the *B. monosperma* samples, maximum amount of phenolic content was recorded as 85.88 mg/100 g in flower extract followed by 64.84 mg/100 g in stem extract and 60.48 mg/100 g in leaves extract (Fig. 3). These variations were found statistically significant when analyzed by DMRT at 5% level of significance. Salar & Seasotiya (2011) reported that antioxidant properties of bark of *B. monosperma* extracted according to increasing and decreasing solvent polarity. According to them significant variations

were found in total phenolic content and antioxidant activity depending on the solvent and method of extraction.

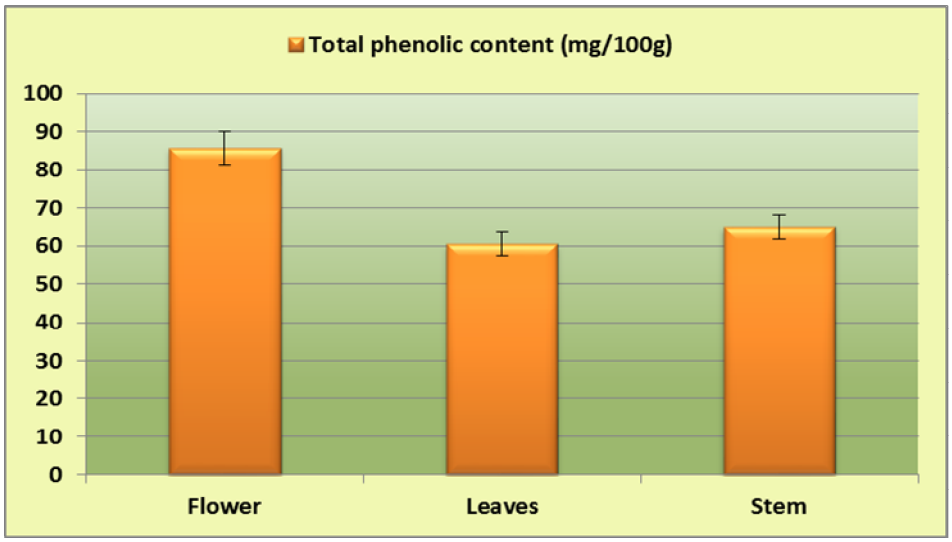

Fig. 3. Total phenolic content (TPC) of methanolic crude extracts (GAE/100 g) of flower, leaves and stems of *B. monosperma*

Phenolic compounds are widely distributed in plants (Li *et al*., 2006), which have gained great attention, due to their antioxidant activities and free radical-scavenging abilities, which potentially have beneficial implications for human health (Govindarajan *et al*., 2007). In the present investigation, other than phenols, stem and leaves extract showed the presence of sterols, steroids, terpenoids and glycosides which are known to have ameliorative effect on human health. Besides, COX-2, an enzyme responsible for inflammation and pain in human being are inhibited by aromatic-5-membered ring heterocycles. The COX-2 inhibitors have analgesic, antipyretic and inflammatory activity comparable to NSAIDs and are used therapeutically in acute pain, and primary dysmenorrhea (Rao *et al*., 2010). Moreover, epidemiological studies and associated meta-analyses strongly suggested that consumption of diets rich in plant polyphenols offered some protection against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases (Pandey & Rizvi, 2009). Thus, the results of the present study may be a proof of a scientific basis for the use of *B. monosperma* in traditional medicine and further studies are needed to focus on isolation of novel bioactive compounds from different parts of *B. monosperma* following extensive phytochemical and pharmacological studies.

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