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Evaluation of three medicinal plants of Bangladesh for antioxidant and cytotoxic potentials

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Abstract

The crude methanol extracts of whole plant of *W. chinensis*, stem bark *of M. diplotricha* and leaves of *B. malabarica* as well as their hexane, carbon tetrachloride, chloroform and aqueous soluble partitionates were subjected to screenings for antioxidant potential in terms of total phenolic content and free radical scavenging activity and brine shrimp lethality bioassay. The antioxidant potential was evaluated using butylated hydroxytolune (BHT) and ascorbic acid as standards. Among the extractives of *W. chinensis*, the crude methanol extract exhibited the highest free radical scavenging activity (IC_{50}= 42.75±0.21 µg/ml) while the aqueous soluble fraction of *M. diplotricha* demonstrated a phenolic content of 30.13±0.87 mg of GAE/g of extractives which could be correlated with its free radical scavenging activity (IC_{50} = 32.46±0.88 µg/ml). The aqueous soluble fraction of leaves of *B. malabarica* revealed the highest free radical scavenging activity (IC_{50} = 62.11±0.47 µg/ml). In the brine shrimp lethality bioassay, among the extractives of *W. chinensis*, the hexane soluble fraction displayed the highest cytotoxic potential (LC_{50}= 73.02±0.36 µg/ml) whereas the aqueous soluble extractives of *M. diplotricha* demonstrated the highest cytotoxic activity (LC_{50}=35.32±0.57 µg/ml). On the other hand, among all the test samples of *B. malabarica*, the methanolic crude extract exhibited the highest cytotoxic potential with LC_{50} value 33.26±0.90 µg/ml. The standard vincristine sulfate exhibited an LC_{50} value of 0.45 µg/ml.

Keywords: *Wedelia chinensis* Osbeck. Merr., *Mimosa diplotricha* Sauvalle., *Bauhinia purpurea* Roxb., free radical scavenging activity, brine shrimp lethality

Introduction

According to the World Health Organization (WHO), 80% of the world's populations rely on traditional medicines (Adamu et al. 2004). The practice of herbal medicine is common in rural areas where western medicines are too expensive or not available (Adamu et al. 2004). Humans have frequently used plants to treat common infectious diseases and some of these traditional medicines are still part of the habitual treatment of various maladies. It has been reported that 115 articles were published on the antimicrobial activity of medicinal plants in Pubmed during the period between 1966-1994, but in the following decade, between 1995 and 2004, 307 were published (Rios and Recio, 2005).

The demand for more and more drugs from plant sources is continuously increasing. It is therefore essential for systematic evaluation of plants used in traditional medicine for various ailments. Hence, there is need to screen medicinal plants for promising biological activity (Chowdhury et al. 2009). Drugs derived from unmodified natural products or drugs semi-synthetically obtained from natural sources corresponded to 78 % of the new drugs approved by the FDA between 1983 and 1994 (Cragg et al. 1997).

Wedelia chinensis Osbeck. Merr. (Synonyms: *Solidago chinensis* Osbeck., *Verbesina calendulacea* L.; Bengali name: Kesraj, Bangra, Bhimraj, Bhimra, Mahavringaraj) commonly known as Chinese Wedelia; is a yellow-flowered perennial herb of sunflower family Asteraceae. In Bangladesh, the plant is found in Chittagong, Dhaka, Mymensingh, Patuakhali, Tangail and Nijum Deep. *W. chinensis* extract has been reported to attenuate androgen receptor activity and orthotopic growth of prostate cancer (Tsai et al. 2009). The essential oil of *W. chinensis* is capable of reducing oxidative stress due to cancer development (Manjamalai and Grace, 2012).

Mimosa diplotricha Sauvalle. (Synonyms: *Mimosa invisa* C. Mart., *Morongia pilosa* Standl.) commonly known as giant sensitive plant, is a shrubby or sprawling annual vine of Fabaceae family. The plant is native to Brazil and is extremely invasive in the Pacific, where it has been introduced on all island groups. 5-Deoxyflavones with cytotoxic activity have been isolated from *M. diplotricha* (Lin et al. 2011).

Bauhinia purpurea Roxb. (Synonyms: *Bauhinia acida* Korth., *Casparea castrata* Hassk. Hassk.; Bengali name: Phutki, Kanchan, Karmai) is an erect low brushy tree of Caesalpiniaceae family. The plant is available in evergreen and deciduous forests of Sylhet in Bangladesh. Seven flavonols, including 6, 8-di-C-methyl kaempferol 3-methyl ether, kaempferol, afzelin, quercetin, isoquercitrin, quercitrin, and hyperoside were isolated from the methanol extract of leaves (Kaewamatawong et al. 2008). The stem bark has been found to possess significant antioxidant activity (Krishnaswamy et al. 2013). Racemosol and demethylracemosol, together with their possible biogenetic precursors, preracemosol A and preracemosol B, were isolated from the roots of *B. malabarica* (Kittakoopa et al. 2000).

As part of our ongoing investigations on medicinal plants of Bangladesh (Sharmin et al. 2013; Chowdhury et al. 2013; Sarker et al. 2014), the crude methanol extracts of whole plant of *W. chinensis,* stem bark *of M. diplotricha* and leaves of *B. malabarica* growing in Bangladesh, as well as their organic and aqueous soluble fractions were studied for antioxidant and cytotoxic activities for the first time and we, here in, report the results of our preliminary investigations.

Materials and methods

Collection of plant materials and extraction:

The whole plant of *W. chinensis,* stem bark *of M. diplotricha* and leaves of *B. malabarica* were collected in March 2012 from Dhaka and voucher specimens for these collections have been deposited in Salar Khan Herbarium, Department of Botany, University of Dhaka.

The collected plant materials were cleaned, sun dried and pulverized. The powdered materials (500 g each) of the collected plants were separately soaked in 2.0 liters of methanol at room temperature for 7 days. The extracts were then filtered through fresh cotton bed and finally with Whatman filter paper number 1 and concentrated with a rotary evaporator at reduced temperature and pressure. An aliquot (5 g) of each of the concentrated methanol extract was fractionated by the modified Kupchan partition protocol (VanWagenen et al. 1993)

and the resultant partitionates were evaporated to dryness with rotary evaporator to yield hexane (HXSF), carbon tetrachloride (CTCSF), chloroform (CSF) and aqueous (AQSF) soluble materials (Table 1). The residues were then stored in a refrigerator until further use.

Table 1: Kupchan partitioning of *W. chinensis, M. diplotricha and B. malabarica.*

Crude extract/ Fractions	*W. chinensis* (g)	*M. diplotricha* (g)	*B. malabarica* (g)
ME	5.0	5.0	5.0
HXSF	1.0	1.3	1.0
CTCSF	1.5	0.8	1.0
CSF	1.0	0.5	0.5
AQSF	0.5	1.5	1.5

ME= Methanolic crude extract; HXSF= Hexane soluble fraction; CTCSF= Carbon tetrachloride soluble fraction; CSF= Chloroform soluble fraction; AQSF= Aqueous soluble fraction

Total Phenolic content:

The total phenolic content of the extractives was determined with Folin-Ciocalteau reagent by using the method developed by Harbertson and Spayd (2006).

DPPH free radical scavenging assay:

Following the method developed by Brand-Williams et al. (1995), the antioxidant activity of the test samples was assessed by evaluating the scavenging activities of the stable 1,1-diphenyl-2-picrylhydrazyl(DPPH) free radical by using synthetic antioxidants, butylated hydroxytoluene (BHT) and ascorbic acid as positive controls.

Brine shrimp lethality bioassay:

This technique was applied for the determination of general toxic properties of the dimethyl sulfoxide (DMSO) solutions of plant extractives against *Artemia salina* in a single day *in vivo* assay (Meyer et al. 1982). Vincristine sulfate was used as positive control.

Statistical analysis

For all bioassays, three replicates of each sample were used for statistical analysis and the values are reported as mean ± SD.

Results and discussion

The crude methanol extracts of whole plant of *W. chinensis*, stem bark *of M. diplotricha* and leaves of *B. malabarica* as well as their hexane, carbon tetrachloride, chloroform and aqueous soluble partitionates were subjected to screenings for antioxidant potential, in terms of total phenolic content and free radical scavenging activity and brine shrimp lethality bioassay.

The test samples of *W. chinensis* exhibited significant free radical scavenging activity with IC_{50} values ranging from 42.75 µg/ml to 177.49 µg/ml. Among the extractives of *W. chinensis*, the crude methanol extract exhibited the highest free radical scavenging activity (IC_{50}= 42.75±0.21 µg/ml) which could be correlated to its phenolic content of 35.01±0.84 mg of GAE/g of extractives while the aqueous soluble fraction of *M. diplotricha* demonstrated a phenolic content of 30.13±0.87 mg of GAE/g of extractives and exhibited the highest free radical scavenging activity among the *M. diplotricha* extractives (IC_{50}=32.46±0.88 µg/ml). In DPPH free radical scavenging assay, the aqueous soluble fraction of leaves of *B. malabarica* revealed the highest free radical scavenging activity (IC_{50}= 62.11±0.47µg/ml) when compared to ascorbic acid (IC_{50}=5.80±0.21µg/ml) (Table 2).

Table 2: Total phenolic content, free radical scavenging and cytotoxic activities of *W. chinensis, M. diplotricha and B. malabarica* extractives.

Plants	Samples/ Standards	Total phenolic content (mg of GAE/g of extract)	DPPH Free radical scavenging activity (IC_{50} µg/ml)	Cytotoxic activity (LC_{50} µg/ml)
W. chinensis	**ME**	35.01±0.84	42.75±0.21	95.02±0.15
	HXSF	13.57±0.84	92.13±0.53	73.02±0.36
	CTCSF	28.20±0.84	50.97±0.81	89.86±0.74
	CSF	12.92±0.26	92.44±0.43	94.63±0.42
	AQSF	3.55±0.23	177.49±0.27	133.64±0.22
M. diplotricha	**ME**	5.67±0.19	87.12±0.17	102.34±0.28
	HXSF	1.67±0.48	296.94±0.84	76.77±0.52
	CTCSF	5.39±0.56	54.55±0.23	45.37±0.37
	CSF	7.20±0.11	55.34±0.45	55.09±0.39
	AQSF	30.13±0.87	32.46±0.88	35.32±0.57
B. malabarica	**ME**	4.97±0.85	140.63±1.07	33.26±0.90
	HXSF	6.08±0.66	355.72±0.02	521.96±1.16
	CTCSF	5.71±0.15	77.26±0.57	435.92±0.53
	CSF	3.01±0.63	268.57±0.32	381.46±0.34
	AQSF	13.99±0.26	62.11±0.47	42.94±0.58
	VS	-	-	0.45±0.43
	BHT	-	27.50± 0.54	-
	Ascorbic acid	-	5.80± 0.21	-

ME= Methanolic crude extract; HXSF= Hexane soluble fraction; CTCSF= Carbon tetrachloride soluble fraction; CSF= Chloroform soluble fraction; AQSF= Aqueous soluble fraction; VS= Vincristine sulfate; BHT= Butylated hydroxytolune.

In the brine shrimp lethality bioassay, the hexane soluble fraction of *W. chinensis* displayed the highest cytotoxic potential having LC_{50} value 73.02±0.36 µg/ml where as among the extractives of *M. diplotricha*, the aqueous soluble fraction demonstrated the highest cytotoxic activity (LC_{50}=35.32±0.57 µg/ml. On the other hand, among all the test samples of *B. malabarica,* the methanolic crude extract exhibited the highest cytotoxic potential with LC_{50} value 33.26±0.90 µg/ml (Table 2).

Conclusion

The objective of the study was to evaluate the antioxidant and cytotoxic potentials of crude methanol extracts of whole plant of *W. chinensis*, stem bark *of M. diplotricha* and leaves of *B. malabarica* as well as their hexane, carbon tetrachloride, chloroform and aqueous soluble partitionates. It is clearly evident from the above findings that the extractives of all the plant extractives revealed mild to moderate antioxidant and cytotoxic potentials. Therefore, these plants are good candidates for further systematic, chemical and biological studies to isolate the active principles.

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