

Original Research Article**FREE RADICAL SCAVENGING ACTIVITY OF SIX MEDICINAL PLANTS OF BANGLADESH: A POTENTIAL SOURCE OF NATURAL ANTIOXIDANT****Shakhawat Hossain¹, Jahidul Islam¹, Firoj Ahmed², Md. Amjad Hossain³, Mohammad Abdul Kaium Siddiki³ and S. M. Moazzem Hossen⁴**

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ABSTRACT

The crude meoh extracts of six medicinal plants of bangladesh (*ipomoea paniculata*, *withania somnifera*, *mikania cordata*, *abroma augusta*, *bombax ceiba*, and *oxalis corniculata*) were screened for primary types of phytochemicals presence and in-vitro anti-oxidant activity by using the 1,1-diphenyl-2-picrylhydrazyl-hydrate (dpph) free radical scavenging assay. Of all of them, the meoh extracts of the leaves of *withania somnifera* and *oxalis corniculata* showed strong antioxidant activity (ic50:27.8 and 19.98 µg/ml), while the meoh extracts of *ipomoea paniculata*, *abroma augusta* and *bombax ceiba* showed moderate activity (ic50: 86.48, 101.4 and 58.6 µg/ml, respectively). Moreover, mild anti-oxidant activity was observed with the meoh extract of *mikania cordata* (ic50 > 300 µg/ml).

Keywords: *Ipomoea paniculata*; *withania somnifera*; *mikania cordata*; *abroma augusta*; *bombax ceiba*; *oxalis corniculata*; antioxidant; DPPH free-radical.

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1. INTRODUCTION

Free radicals are metastable chemical species which are generated as by-products of various biochemical reactions *in vivo*. These radicals can be emerged as molecular sharks, which if not scavenged effectively on time, are capable of damaging crucial bio-molecules present in cell membranes, mitochondria, DNA, etc. and thus alter various pathophysiological conditions. The role of free radicals, also called 'reactive oxygen species' (ROS), has been well-identified in the pathogenesis of many disease conditions such as rheumatoid arthritis, hemorrhagic shock, cardiovascular disorders, cystic fibrosis, neurodegenerative diseases (e.g. Parkinsonism, Alzheimer's disease), gastrointestinal ulcerogenesis, AIDS and even early senescence [1,2]. ROS is a collective term, which includes not only the oxygen radicals (O₂^{·-} and ·OH) but also some non-radical derivatives of oxygen. These include hydrogen peroxide (H₂O₂), hypochlorous acid (HOCl) and ozone (O₃) [3].

Search for compounds or preparations with antioxidant activities, has thus become an important strategy for the treatment of such ROS generated illness. Several plant extracts and some phytochemicals have been found to have quite prominent antioxidant activity [4-6]. The objective of the present study was to investigate the antioxidant activity of the crude extracts of different parts of six plant which are available in Bangladesh, namely *Ipomoea paniculata* (Root), *Mikania cordata* (Bark), *Withania somnifera* (Root), *Abroma augusta* (Leaves), *Bombax ceiba* (Root), and *Oxalis corniculata* (Whole plant). The botanical and ethno pharmacological features (along with the parts used in extractions) of the plants are enlisted in Table 1.

2. MATERIALS AND METHODS**2.1 Plant Materials**

All plants were collected from Natore Medicinal plant village in the month of March, 2013 and were authenticated by Mr. Md. Mustafizur Rahman, Lecturer, Department of Botany, Rajshahi University.

2.2 Extraction of Plant Materials

Selected plants are *Ipomoea paniculata* (Root), *Mikania cordata* (Bark), *Withania somnifera* (Root), *Abroma augusta* (Leaves), *Bombax ceiba* (Root), and *Oxalis corniculata* (Whole plant). All leaves, root, barks collected from mature tree. Plant parts are collected, cleaned and dried inside the laboratory and finally dried at 40°C inside the oven. From each plant part, about 600 g of powdered plant material was taken in a clean, flat bottomed glass container (4 L) and soaked in 1300 mL methanol as solvent.

Table 1. The botanical and ethnopharmacological features of the selected plants

Plants	Type	Traditional use	Chemical constituents	Reported bioactivity	Part(s) used (solvent)
<i>Ipomoea paniculata</i> (Convolvulaceae)	Perrenial climber	Uterine pain, sexual disability, infertility, gastric ulcer, ulcerative colitis, etc.[7].	Alantolactone, sitosterol, inunolide [8] ; glycoside paniculatin [9].	Stimulant effect on the myocardium and respiration [9]; hypotensive effect [10].	Tuber (MeOH) (Yield: 10%)
<i>Withania somnifera</i> (Solanaceae)	Shrub	Aphrodisiac, sedative, rejuvenative and life prolonging properties [11].	Tropane, tigloyxtropine, choline, anaferine, pelletierine, isopelletierine, anahygrine, and cuscohygrine [12].	Adaptogen, antibiotic, abortifacient, aphrosidiac, astringent, antiinflammatory, deobstruent, diuretic, narcotic [13].	Roots (MeOH) (Yield: 12%)
<i>Mikania cordata</i>	Perrenial creeper	Treatment of dyspepsia, dysentery. anti-carcinogenic [14].	Sesquiterpene dilactones, deoxy mikanolide and scandenolid [15,16].	Antimicrobial, analgesic [16]. Antiulcer [17]. Anticarcinogenic [14].	Whole plant (Methanol) (Yield: 16%)
<i>Abroma augusta</i> (Malvaceae)	Tree	Uterine disorders, gonorrhoea, skin problems, dysmenorrhoeal [18].	Alkaloids, triterpenes, flavonoids, megastigmanes, benzohydrofurans and their glycosides and phenylethanoid glycoside[19].	Hypoglycemic [20], antioxidant [21] anti-inflammatory [22].	Leaves (Methanol) (Yield: 14%)
<i>Bombax ceiba</i> (Malvaceae)	Tree	Stimulant, haemostatic, aphrodisiac, diuretic, antidiarrhoeal, cardiogenic, emetic, demulcent, antidysenteric, and antipyretic [23,24].	cholesterol, stigmasterol, campesterol and a-amyrin, hydrocarbons, seven flavones: isohemigossypol-1-methyl ester, acid lactone, bombaxquinone, lacinilene C, sesquiterpene lactone	Antioxidant, hypoglycemic, analgesic, antibacterial, hepatoprotective [25].	Roots (Methanol) (Yield: 11%)
<i>Oxalis corniculata</i> (Oxalidaceae)	Herb	The plant is cooling, antiseptic, astringent, appetiser and antiscorbutic, useful in fevers and biliousness, juice of the plant cures scurvy, piles, anaemia and tympanites, dysentery, improves appetite and digestion [26].	Glycoside, ascorbic acid, carotene, isovitexin, citric acid, etc. [27].	Antibacterial [28]; Cardio protective and antioxidant [29].	Whole plant(Methanol) (Yield: 12%)

2.3 Chemicals

Used chemicals like Ascorbic acid collected from Loba, India and DPPH (2,2-Diphenyl-1-picrylhydrazyl) from Aldrich, USA.

2.4 Phytochemical screening

All of the crude extracts were qualitatively analyzed for the presence of different chemical groups, such as alkaloids, glycosides, steroids, tannins, flavonoids and saponins [31].

2.5 Screening for Antioxidant Activity

Antioxidant activity of the extracts was determined on the basis of the modified method of Gupta *et al.* [32]. The free radicals scavenging activity of all extract were measured by decreased in the absorbance of EtOH solution of DPPH (2,2-Diphenyl-1-picrylhydrazyl). Stock solutions (1 mg/ml) of the plant extracts were prepared in EtOH from which serial dilutions were carried out to obtain concentrations of 1, 5, 10, 50, 100 and 500 µg/ml. Diluted solutions (2 ml) were added to 2 ml of a 0.004% ethanol solution of DPPH, mixed and allowed to stand for 30 min for reaction to occur. The absorbance was determined at 517 nm and from these values corresponding percentage of inhibitions were calculated. Then % inhibitions were plotted against concentration and from the graph IC₅₀ was calculated. The experiment was performed in triplicate and average absorption was noted for each concentration. Ascorbic acid was used as positive control.

3. RESULTS

3.1 Preliminary Phytochemical Analysis

Results of phytochemical functional group tests are tabulated in Table 2. Flavonoid one of the phytochemicals gives +ve results for all plant samples. Glycosides present in all samples excepting *Ipomoea paniculata*.

Table 2. Results of Phytochemical Screening of the selected plants

Plant Extract	Alkaloid	Tannin	Glycosides	Flavonoid	Saponin	Steroid
<i>Ipomoea paniculata</i>	+	-	-	+	-	+
<i>Withania somnifera</i>	+	+	+	+	+	-
<i>Mikania cordata</i>	-	+	+	+	+	+
<i>Abroma augusta</i>	+	-	+	+	-	-
<i>Bombax ceiba</i>	+	+	+	+	+	-
<i>Oxalis corniculata</i>	-	+	+	+	+	-

+: Positive result; -: Negative result;

3.2 Antioxidant Activity Study

Antioxidant activities of the extracts were tested using the DPPH free radical scavenging assay. Results indicated that the MeOH extract of *Withania somnifera* and *Oxalis corniculata* showed strong antioxidant activity, IC₅₀:27.8 and 19.98 µg/ml, respectively and on the other hand *Ipomoea paniculata*, *Abroma augusta* and *Bombax ceiba* showed moderate activity like IC₅₀: 86.48, 101.4 and 58.6 µg/ml, respectively. All results are summarized in Table 3.

Table 3. Evaluation of antioxidant activity of the collected plants

Sample	Concentration ($\mu\text{g/ml}$)	% inhibition	IC ₅₀ ($\mu\text{g/ml}$)
MeOH extract of <i>I. paniculata</i>	1	1.44 \pm 0.01	86.48
	5	9.17 \pm 0.01	
	10	15.33 \pm 0.001	
	50	42.24 \pm 0.025	
	100	45.93 \pm 0.018	
	500	83.97 \pm 0.01	
MeOH extract of <i>W. somnifera</i>	1	28.66 \pm 0.011	27.80
	5	37.79 \pm 0.013	
	10	43.86 \pm 0.011	
	50	45.67 \pm 0.035	
	100	47.75 \pm 0.028	
	500	86.15 \pm 0.041	
MeOH extract of <i>M. cordata</i>	1	0.94 \pm 0.016	>300
	5	2.20 \pm 0.017	
	10	3.99 \pm 0.019	
	50	9.98 \pm 0.045	
	100	24.31 \pm 0.038	
	500	56.21 \pm 0.051	
MeOH extract of <i>A. augusta</i>	1	10.24 \pm 0.013	101.4
	5	27.98 \pm 0.019	
	10	40.49 \pm 0.021	
	50	46.37 \pm 0.055	
	100	47.61 \pm 0.038	
	500	58.57 \pm 0.047	
MeOH extract of <i>B. ceiba</i>	1	6.54 \pm 0.015	58.60
	5	16.85 \pm 0.012	
	10	24.57 \pm 0.011	
	50	47.24 \pm 0.035	
	100	58.58 \pm 0.028	
	500	77.73 \pm 0.019	
MeOH extract of <i>O. corniculata</i>	1	6.54 \pm 0.015	19.98
	5	16.85 \pm 0.012	
	10	24.57 \pm 0.011	
	50	47.24 \pm 0.035	
	100	58.58 \pm 0.028	
	500	77.73 \pm 0.019	
Ascorbic acid	1	5.71 \pm 0.001	7.95vxcz
	5	38.4 \pm 0.002	
	10	69.3 \pm 0.001	
	50	92.09 \pm 0.001	
	100	92.58 \pm 0.004	
	500	95.44 \pm 0.001	

Values are expressed as mean \pm S.D; Number of Trail, n =3

4. DISCUSSION

DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of a compound, drug, crude drug or a plant extract. In the present study, it was noticed that the MeOH extracts

of *Withania somnifera* and *Oxalis corniculata* showed strong antioxidant activity, while MeOH extracts *Ipomoea paniculata*, *Abroma augusta* and *Bomba ceiba* showed moderate activity. Remaining MeOH extract of *Mikania cordata* showed mild free-radical scavenging activity. The free radical scavenging property may be one of the mechanisms by which this drug is effective in traditional medicine.

Previous demonstrations and research are evident that the tannins and flavonoids are phenolic compounds and responsible for antioxidant properties of many plants [4]. In the present experiment, all plant extract contain flavonoid and tannin and all the plants extract showing antioxidant property, this nature may be due to tannins and flavonoids. The result of all plant extract may be correlating with their antioxidant activity against DPPH free radical [33].

Antioxidants are drugs used for different diseases like aging, alzheimer's diseases and many other diseases due to oxidation in human body. Antioxidants are responsible for scavenging of free radicals from body. The free radical scavenging property may be one of the mechanisms by which these plant parts are effective in their ethno-pharmacological uses against different ailments. Further studies, like extraction in different solvents, comprising of thorough phytochemical investigations of the used plants and evaluation for anti-oxidant activity using other models (e.g. various biochemical assays, both *ex vivo* and *in vivo*) are essential to characterize them as biological antioxidants.

5. CONCLUSION

Withania somnifera and *Oxalis corniculata* are two potential plants having strong antioxidant activity. In future they may be good source of antioxidant agent and may be useful for human being.

ETHICAL APPROVAL

"All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki."

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Halliwell H. Free radicals, antioxidants, and human disease: Curiosity, cause or consequence. *Lancet* 1994;344:721–724.
2. Halliwell B, Gutteridge JMC. Free radicals. In *Biology and Medicine*, 2nd ed., Clarendon Press, Oxford. 1985;279–315.
3. Bandhopadhyay U, Das D, Banerjee RK. Reactive oxygen species: oxidative damage and pathogenesis. *Curr. Sci.* 1999;77:658–666.
4. Larson RA. The antioxidants of higher plants. *Phytochemistry* 1988;27(4):969–978.

5. Tripathi YB, Chaurasia S, Tripathi E, Upadhyay A, Dubey GP. *Bacopa monniera* Linn. As an antioxidant: Mechanism of action. *Ind. J. Exp. Biol.* 1996;34:523–526.
6. Vani T, Rajani M, Sarkar S, Shishoo CJ. Antioxidant properties of the Ayurvedic formulation Triphala and its constituents. *Int. J. Pharmacognosy* 1997;35(5):313–317.
7. Yusuf M, Begum J, Hoque MN, Chowdhury JU. Medicinal Plants of Bangladesh. BCSIR, Chittagong.2009:794
8. Ono M, Fukuda H, Murata H, Miyahara K. Resin glycosides from the leaves and stems of *Ipomoea digitata*. *J. Nat. Med.*, 2008;63:176-180.
9. Matin MA, Tewari JP. et al.,. Pharmacological effects of paniculatin a glycoside isolated from *Ipomoea digitata*. *Journal of Pharmaceutical Sciences* 2006;58:757–759.
10. Jain V, Verma SK, Katewa SS. Therapeutic validation of *Ipomoea digitata* tuber for its effect on cardio-vascular risk parameters. *Ind. J. Traditional Knowledge* 2011;10:617-623.
11. Nadkarni, K.M. *Indian Materia Medica*. Vol. I, 1976.
12. Atta-ur-Rahman et al., Two New Withanolides from *Withania Somnifera*," *Heterocycles*, 1992; Vol. 34:No. 4:689-698.
13. Panda S, Kar A. Evidence for free radical scavenging activity of *Ashwagandha* root powder in mice. *Indian J Physiol Pharmacol* 1997;41:424-426.
14. Bishayee A & M, C. Anticarcinogenic biological response of *Mikania cordata*: reflections in hepatic biotransformation systems. *Cancer Lett.* 1994;81:193-200.
15. Ysrael, M.C. and K.D. Croft. Inhibition of leukotriene and platelet activating factor synthesis in leucocytes by the sesquiterpene lactone scandenolide. *Planta Med.* 1990;56: 268-270.
16. Ahamed M, Rahman M, Alimuzzaman M & JA, S. Analgesic sesquiterpene dilactone from *Mikania cordata*. *Fitoterapia* 2001;72.
17. Paul RK, Jabbar A & MA, R. Antiulcer activity of *Mikania cordata*. *Fitoterapia*, 2000;71:701-703.
18. Prajapati ND, Purohit SS, Sharma AK, Kumar T. *A hand book of medicinal plants*, A complete source book. Agrobios (India) Publisher; Jodhpur. 2003.
19. Gupta B, Nayak S, Solanki S. *Abroma augusta* Linn: A review. *Der Pharmacia Sinica* 2011; 2(4): 253-61.
20. Halim EM, Hussain A. Hypoglycemic, hypolipidemic and antioxidant properties of combination of *Curcumin longa* Linn. and partially purified product from *Abroma augusta* L. in Streptozotocin induced diabetes. *Indian J Clin Biochem* 2002;17(2): 33-43. <http://dx.doi.org/10.1007/BF02867969>
21. Nahar, L., Ripa, F.A., Rokonuzzaman, M. & Al-Bari, M.A.A. Investigation on antioxidant activities of six indigenous plants of Bangladesh. *Journal of Applied Sciences Research* 2009;5(12): 2285–2288.
22. Sutapa Das, Rana Datta, and Subhangkar Nandy, Phytochemical screening and evaluation of anti-inflammatory activity of methanolic extract of *Abroma augusta* Linn, *Asian Pacific Journal of Tropical Disease*.2012;S114-S117
23. Williamson EM, *Major Herbs of Ayurveda*, (Churchill Livingstone Publishers), 2002, 261.

24. Singh MP & Panda H, Medicinal Herbs and their formulations, Vol I, (Daya Publishing House, New Delhi), 2005;176-78.
25. Pankaj H. Chaudhary, Somshekhar S. Khadabadi. Bombax ceiba Linn. : Pharmacognosy, Ethnobotany and Phyto-pharmacology, Pharmacognosy Communications, 2012; 2(3):02-09. doi:10.5530/pc.2012.3.2
26. Yusuf M, Begum J, Hoque MN, Chowdhury JU., Medicinal Plants of Bangladesh. BCSIR, Chittagong. 2009.
27. Hiroki MizoKami, Kaoritomita-yokotani, Kunijiro Yoshitama. Flavanoids in the leaves of *oxalis corniculata* and sequestration male grass blue butterfly, pseudozizeeria maha. *J Plant Res.* 2008; 121:133-136.
28. Raghvendra MP, Satish S. Raveesha KA. Phytochemical analysis and antibacterial activity of *Oxalis Corniculata*, a known medicinal plant. *My Science.* 2006;1:72-78
29. Abhilash P.A, Nilasha, Prathapan A, Suresh V. Nampoothiri, Lizocherian O, Sunitha T.K, Raghu K.G. Cardio protective effects of aqueous extract of *Oxalis corniculata* in experimental myocardial infarction. *Experimental and Toxicological Pathway.* 2011; 63:535-540.
30. Taylor RSL, Edel F, Manandhar NP, Towers GHN: Antimicrobial activity of Southern Nepalese medicinal plants. *J Ethnopharmacol* 1996, 50:97–102.
31. Trease GE, Evans WC: *Pharmacognosy*. 11th edition. Brailliar Tiridel, Canada: Macmillian Publishers; 1989.
32. Gupta M, Mazumdar UK, Sivahkumar T, Vamis MLM, Karki S, Sambathkumar R, Manikandan L. Antioxidant and anti-inflammatory activities of *Acalypha fruticosa*. *Nig. J. Nat. Prod. Med.* 2003;7:25-29.
33. Sadhu, S.K., Okuyama, E., Fujimoto, H., Ishibashi, M. 2003. Separation of *Leucas aspera*, a medicinal plant of Bangladesh, guided by Prostaglandin inhibitory and Antioxidant activities. *Chem. Pharm. Bull.* 2003;51 (5): 595-98.