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FREE RADICAL SCAVENGING ACTIVITIES OF SOME INDIGENOUS PLANTS OF BANGLADESH

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Abstract: *Four indigenous plant, Bryophyllum daigremontianum (Raym.), Coccinea cordifolia (Linn.), Litsea glutinosa (Lour.) and Micromelum minutum (G. Forst.) have been investigated for their antioxidant activity. The extractives were subjected to assay by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) for evaluation of free radical scavenging property. The IC_{50} value of the organic extracts ranged from 336.45 to 23.85 µg/ml. The aqueous soluble fraction of L. glutinosa demonstrated potent free radical scavenging activity (IC_{50}=23.85µg/ml) as compared to tert-butyl-1-hydroxytoluene (standard) that showed an IC_{50} value of 34.89 µg/ml. The results primarily suggest the presence of potent oxidation inhibitory principles in the leaves of L. glutinosa.*

Keywords: *1, 1-diphenyl-2-picrylhydrazyl (DPPH), antioxidant, free radical scavenger*

Introduction

There is an increased evidence for the participation of free radicals in the etiology of various diseases like cancer, diabetes, cardiovascular diseases, autoimmune disorders, neurodegenerative diseases and aging, etc[1]. A free radical is defined as any atom or molecule possessing unpaired electrons. Free radicals can cause a wide range of toxic oxidative reactions like initiation of the peroxidation of the membrane lipids leading to the accumulation of lipid peroxides, direct inhibition of mitochondrial respiratory chain enzymes, fragmentation or random cross linking of molecules like DNA, enzymes and proteins which ultimately leads to cell death [2]. Antioxidants are agents which scavenge the free radicals and prevent the damage caused by them. They can greatly reduce the damage due to oxidants by neutralizing thefree radicals before they can attack the cells and prevent damage to lipids, proteins, enzymes, carbohydrates and DNA [3]. A wide range of antioxidants from both natural and synthetic origin has been proposed for use in the treatment of various human diseases [4]. Flavonoids and other phenolic compounds of plant origin have been reported as scavengers of free radicals [5]. Hence, nowadays search for natural antioxidant source is gaining importance in the discovery of new drug candidates.Extensive literature survey has revealed that no research has been conducted on the antioxidant activities of the selected plants (http:/ncbi.nlm.nih.gov/pubmed). As a part of our effort directed to the search for such molecules from natural sources, we studied the the methanol extracts of *B. daigremontianum*(Bengali Name-Pathorkuchi), *C. cordifolia*(Bengali Name-Telakucha),

L. glutinosa(Bengali Name-Mendapata) and *M. minutum,* and the extractives generated from these. We, herein, report the results of our preliminary investigation.

Chemicals and Reagents: Folin-Ciocalteu reagent, anhydrous sodium carbonate, 2,6-di-tertbuytl-4-methylphenol (BHT), 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma chemical Co. USA. Deionized water was obtained from the faculty of Pharmacy, University of Dhaka.

Materials and Methods

Plant Materials: The leaves of *Bryophyllumdai gremontianum* (Family: Crassulaceae), *Coccineacordifolia*,(Family:Cucurbitaceae),*Litseaglutinosa*(Family:Lauraceae)&*Microm elumminutum* (Family: Rutaceae) were collected from Dhaka, Bangladesh, in June, 2009. The voucher specimens for these collections have been deposited in Bangladesh National Herbarium, Mirpur, Dhaka. The leaves of the plants were first separated from the plant, cut into small pieces and air-dried for several days. The pieces were then oven dried in an oven for 24 hours at considerably low temperature to facilitate grinding.

Extraction: The sun dried and powdered leaves of 500 gm of each plant were separately extracted with methanol (500 ml) for 15 days at room temperature with occasional shaking and stirring. It was then filtered through a cotton plug followed by Whatman filter paper number 1. The extracts were concentrated with a rotary evaporator at low temperature (40-45 °C) and reduced pressure. Subsequent evaporation of solvents afforded extract of *B. daigremontianum*(7.3 g),*C.cordifolia*, (6.5 g), L. *glutinosa*(5.2 g), and *M. minutum*(6.9 g).An aliquot (5 gm) of the concentrated methanolic extract from each plant was partitioned by the modified Kupchan method[6] to provide *n*-hexane (HSP), carbon tetrachloride (CTP), chloroform (CSP) soluble fractions. The aqueous (ASP) soluble fraction of each plant was also used for the experimental purpose.

Antioxidant activity

Qualitative analysis: The methanol extract was applied on a TLC plate as a spot (100 µg/ml) for chromatographic separation of the extract using the mobile phase chloroform-methanol (95:5, v/v). After development of the chromatogram, the plate was sprayed with DPPH (0.15 % w/v) solution using an atomizer. The changes of color on the TLC plate (yellowish on pinkish background) were noted as an indicator of the presence of antioxidant substances.

Quantitative analysis: The free radical scavenging activity the plant extractives on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was estimated by the method established by Brand-Williams *et al.* 2.0 ml of a methanol solution of the sample (extractive/ standard) at different concentration (500 µg/ml to 0.977 µg/ml) were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml). After 30 min of reaction at room temperature in dark place the absorbance was measured at 517 nm against methanol as blank by a UV spetrophotometer. Then the inhibition of free radical DPPH in percent (I%) was calculated as follows:

(I%) = (1 – A_{sample}/A_{blank}) × 100, where A_{blank} is the absorbance of the control reaction (containing all reagents except the test material).Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted by inhibition percentage against extractive/standard concentration.

Statistical analysis: Three replicates of each sample were used for statistical analysis and the values are reported as mean ±SD.

Results and Discussion

The extractives of *B. daigremontianum, C cordifoli, L. glutinosa*and *M. minutum,* were assessed for free radical scavenging activity and the results are presented in Table-1. The extractive demonstrated varying degrees of antioxidant activity. In this study, the aqueous soluble fraction of methanol extract of *L. glutinosa*was found to exhibit the highest antioxidant activity with an IC_{50} value of 23.85 μg/ml followed by carbon tetrachloride soluble fractions of *B. daigremontianum* with IC_{50} value of 40.50μg/ml, chloroform soluble fractions of *L. glutinosa* with IC_{50} value of 50.65μg/ml, aqueous soluble partitionate of *M. minutum* with IC_{50} value of 53.34μg/ml. On the other hand, moderate antioxidant activity was revealed by hexane soluble fraction of *B. daigremontianum,* carbon tetrachloride fractions of *C. cordifolia*andcarbon tetrachloride soluble fraction of *M. minutum.* The other fractions demonstrated weak free radical scavenging activity. The antioxidants act either by scavenging various types of free radicals derived from oxidative processes, by preventing free radical formation through reduction precursors or by chelating metals [7, 8, 9]. The reduction of DPPH assay has been used to detect products with antioxidant activity as free radical scavengers [10, 11].

Conclusion

From the above results, it can be concluded that all the plants have great potential to act as antioxidant, which also indicates the presence of secondary metabolites having antioxidant actrivities. These plants could be subjected to extensive chromatographic separation and purification processes to isolate the bioactive compounds for the discovery of lead molecule.

Table 1: Amount of different fractions of methanolic extract

Fraction of methanolic extract	Amount of fractions (mg)			
Plant	*B. daigremontianum*	*C.cordifolia*	*L. glutinos*	*M. minutum*
n-Hexane soluble fraction	450	550	400	480
CCl_4 soluble fraction	325	375	380	360
$CHCl_3$ soluble fraction	250	220	220	280

Table 2. Free radical scavenging activities of test samples

Samples (Different soluble fractions of methanolic extract)	IC_{50} (µg/ml)				
	Standard	Plant			
	Tert-butyl-1-hydroxy-toluene	*B. daigremontianum*	*C.cordifolia*	*L. glutinosa*	*M. minutum*
	34.89				
Crude methanolic extract		336.45	249.52	148.43	154.94
Hexane soluble fraction		107.63	352.38	156.63	285.25
Carbon tetrachloride soluble fraction		40.50	125.25	175.35	115.58
Chloroform soluble fraction		204.57	105.25	50.65	264.49
Aqueous fraction		260.65	307.35	23.85	53.34

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