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Phenolics Content and Antioxidant activity of Crude Extract of *Oldenlandia corymbosa* and *Bryophyllum pinnatum*

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ABSTACT

Oldenlandia corymbosa (Rubiaceae) and *Bryophyllum pinnatum* (Crassulaceae) are two common medicinal plants widely distributed in North East India, and used many ethnic group of people in traditional system of medicines for different purposes. The antioxidant potential of the crude methanolic extracts of both the plants were evaluated using DPPH free radical scavenging method and hydroxyl radical scavenging method. IC_{50} value for DPPH free radical scavenging activity of MEO and MEB was found 729.91 and 774.74 mg/gm respectively. IC_{50} value for H_2O_2 scavenging activity of MEO and MEB was found 705.38 and 632.31 mg/gm respectively whereas for standard ascorbic acid it was 583.949 and 56.18 mg/gm respectively. The polyphenolics content of MEO and MEB were found 15.6 and 63.3 mg/gm (gallic acid equivalent).

Keywords: Reactive oxygen species, Oxidative stress, Antioxidant, Polyphenolics, *Oldenlandia corymbosa, Bryophyllum pinnatum*.

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INTRODUCTION

Reactive oxygen species (ROS) are generated in biological system as natural byproduct of the normal metabolism of oxygen and play important roles in cell signaling and homeostasis at lower concentration for maintaining normal functioning of cells.[1] However, during the time of environmental stress, ROS levels can increase dramatically resulting into significant damage to cell structures and functional ability. This cumulates into Oxidative stress, a situation which is occurred as a result of imbalance between the generation of ROS and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage.[2] Such Cellular imbalance leads in to various forms of damage of micro molecules and macromolecules and finally contributes into the manifestation of disease e.g. Sickle Cell Disease, atherosclerosis, Parkinson's disease, heart failure, myocardial infarction, Alzheimer's disease, schizophrenia, bipolar disorder etc. [3]

Biological systems inherently have antioxidant system to neutralize the free radicals which includes antioxidant enzyme system (AOEs) consisting of Superoxide dismutase (SOD) Catalase, Glutathione-S-Transferase (GST), Glutathione Peroxidase (GPx), Glutathione etc. These biological AOEs function as cascade manner to neutralize or eliminate the ROS. The failure of such functioning contributes the diseases manifestation. [4]

In order to neutralize ROS, antioxidants have been supplemented. For potential source of antioxidants natural products are considered as best sources and several botanicals along with their constituents such as polyphones, flavonoids, and tannins have been studied and some antioxidants have been formulated.[5,6] Synthetically also BHT, BHA are developed as antioxidants. However, in real biological state of radical scavenging and subsequent reduction of disease manifestation is still promising research area. [7-9]

Oldenlandia corymbosa (family Rubiaceae) and *Bryophyllum pinnatum* (family crassulaceae) are two herbs commonly found in North East India. These plants have been used by many ethnic group of people from this region as a source of various forms of ailments. Traditionally Oldenlendia corymbosa is used in various hepatic disorders, urinary disorder, Jaundice, Fever, diarrhoea , bilious infection etc. [10,11] Similarly leaves of *Bryophyllum pinnatum* is used in wounds, bruises, boils, jaundice, snakebite, dysentery, urinary trouble, kidney stone and quick healing of wounds. [10,12] However, scientific validation of these two plants is limited. Hence, this work aimed into the radical scavenging property of methanolic extract of the plants using DPPH as a source of radicals.

MATERIALS AND METHODS

Plant materials: collection and preliminary processing

The whole plant Oldenlendia corymbosa and leaves of *Bryophyllum pinnatum* were collected from vicinity of Dibrugarh University campus, Dibrugarh, Assam (India) in the month of January-February, 2008. Plant materials were dried at shade and grinded into coarse powder

by using mechanical grinder. The coarse powders were packed in sealed bags and stored at room temperature in low humidity condition.

Preparation of Extract:

About 20 gm of grinded plant powders were taken and macerate with 200 ml methanol for 2 days with intermittent shaking. After 2 days, it was filtered and the solvent was recovered by using distillation and the extract is concentrated on water bath. The extracts were named as MEO and MEB for Oldenlendia corymbosa and *Bryophyllum pinnatum* respectively.

Determination of total polyphenolic compounds: [13]

Antioxidant compound generally contain phenolic group and hence the amount of phenolic compound of the extracts were estimated by using Folin-Cioclateu reagent, using Gallic acid as a standard phenolic compound. In brief 1 mL of sample solution in methanol was mixed with 5 ml Folin ciolateu reagent and 4 ml sodium carbonate. After shaking it was kept for 30 minute at 20^0C and the absorbance was taken at 765 nm. Using standard curve of Gallic acid, the total phenolic compound content was calculated and expressed as gallic acid equivalent in mg/g of extract.

$$C = \frac{c \times v}{m}$$

C = the content of phenolic group mg/g plant extract in Gallic acid
c = the concentration of gallic acid established from the Calibration curve.
v = the weight of methanolic extract in tubes.
m=the weight of pure plant methanolic extract.

DPPH free radical-scavenging activity: [14]

To determine the antioxidant activity of the extracts, a method based on the reduction of a purple-colored stable free radical DPPH into the yellow-colored diphenylpicryl hydrazine was employed. In brief, 1 mL of methanolic solution of DPPH (0.1 mM,) was incubated with 3 ml of different concentration of the extract at room temperature (25^0C) for 30 minutes. After incubation, the absorbances of the sample were recorded at 490 nm. Decreases in the absorbance of the DPPH indicate increase in the DPPH radical scavenging activity. For each concentration, the assay was run in triplicate. Ascorbic acid solution was used as a standard. Ic_{50} values (concentration required to scavenge 50 % of the free radical) for both Ascorbic acid and the leaf extract were determined. The radical scavenging activity of the tested samples was expressed as an inhibition percentage (IP)

$$\text{DPPH scavenged (\%)} = A_{DPPH}\text{-}A_{test}/A_{DPPH}) \text{ X } 100$$

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Where A_{DPPH} is the absorbance of the 0.1 mM of DPPH solution and A_{test} is the absorbance in the presence of the extract or Ascorbic acid.

Hydrogen peroxide radical scavenging assay: [15]

The capacity of Methanolic extract of *Bryophyllum pinnatum* and *Oldenlandia corymbosa* to inhibit hydrogen peroxide was determined. A solution of Hydrogen peroxide (20mM) was prepared in phosphate buffer solution (PBS, pH 7.4). Various concentration of 1ml of the extract/standard (ascorbic acid) in methanol were added to 2ml of H_2O_2 solution in phosphate buffer solution .The absorbance of hydrogen peroxide was measured at 230nm, after 10 minutes against a blank solution that contained phosphate buffer without hydrogen peroxide .The percentage of H_2O_2 scavenging of both the extracts and standard compound were calculated.

$$\text{Percentage scavenged } (H_2O_2) = \left[\frac{A_o - A_t}{A_o}\right] \times 100$$

Where A_o is absorbance of the control and absorbance of the presence of Methanolic extract of *Oldenlandia corymbosa* and *Bryophyllum pinnatum*.

Statistical Analysis:

All data on all antioxidant activity tests are the average of triplicate analyses. The data were recorded as mean ±SD.

RESULTS

Table 1: In vitro antioxidant activity of Oldenlendia corymbosa and *Bryophyllum pinnatum*

Sl No.	Method		In Gallic acid equivalent	
			Methanolic extract of Oldenlendia Corymbosa (mg/g)	Methanolic extract of *Bryophyllum pinnatum* (mg/g)
1.	Total phenol Compound		15.6	63.3
IC_{50} values in µg/ml				
	Method	Std. Ascorbic acid	Methanolic extract of Oldenlendia Corymbosa (mg/g)	Methanolic extract of Bryophyllum pinantum (mg/g)
2.	H_2O_2	583.949	729.91	774.74
3	DPPH	56.18	705.38	632.31

Total phenolic compound:

The total phenolic compounds of both the plants were expressed as gallic acid equivalent in mg/g of extract. The study revealed that 1 gm of methanolic extract of

Bryophyllum pinnatum and Oldenlendia corymbosa contain 63.3mg and 15.6 mg respectively of gallic acid equivalent which is summarized in (table 1)

H_2O_2 radical scavenging activity:

IC_{50} of the standard Ascorbic acid solution was found to be 583.949 µg/ml, while it was 729.91 µg/ml for Oldenlendia corymbosa and 774.74 µg/ml for *Bryophyllum pinnatum* (table 1)

DPPH free radical scavenging activity:

IC_{50} value for the standard Ascorbic acid was found to be 56.18 µg/ml whereas IC_{50} for Oldenlendia corymbosa and Bryophyllum pinnata were found to be 705.38 µg/ml and 632.31 µg/ml respectively (table 1).

DISCUSSION

Although oxygen is essential for life, its transformation to reactive oxygen species (ROS) may provoke uncontrolled reactions. Such challenges may arise due to exposure to radiation, chemicals, or by other means. Antioxidants may offer resistance against the oxidative stress by scavenging free radicals, inhibiting lipid peroxidation and some other mechanisms.[16]

Phenols are very important plant constituents because of their free radical scavenging ability due to their hydroxyl group.[17] The phenolic compounds may contribute directly to antioxidant action. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in human beings .[18] Our study result shows that the methanolic extract of Oldenlendia corymbosa and *Bryophyllum pinnatum* are found to contain phenolic compound in significant amount, which attributes to its rationality of possessing antioxidant activity.

The stable DPPH radical model is a widely used, relatively quick method for the evaluation of free radical scavenging activity. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. DPPH is a stable free radical that accepts electron or hydrogen radical to become a stable diamagnetic molecule .[19] The absorption maximum of a stable DPPH radical in methanol was at 517 nm. The decrease in absorbance of DPPH radical caused by antioxidant, because of the reaction between antioxidant molecules and radical progresses, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change is color from purple to yellow. Hence DPPH is usually used as a substrate to evaluate the antioxidative activity of antioxidant. [20] It has been reported that oxidative stress, which occurs when free radical formation exceeds the body's ability to protect itself, forms the biological basis of chronic conditions such as arterioselerosis. [21] Our study results show that Oldenlendia corymbosa and *Bryophyllum pinnatum* are free radical inhibitors or scavengers, as well as primary antioxidant that react the free radicals.

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H_2O_2 is weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with the Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects. [22] Hydrogen peroxide is highly important because of its ability to penetrate biological membranes. H_2O_2 itself is not very reactive, but it can sometimes be toxic to cell because of it may give rise to hydroxyl radical in the cells. Thus removing of H_2O_2 is very important for protection of food system. Scavenging of H_2O_2 by Oldenlendia corymbosa and *Bryophyllum pinnatum* may attribute to their phenolic compound, which can donate electron to H_2O_2 thus naturalizing it to water.[23] The difference in H_2O_2 scavenging capacity may be attributed to the structural features of their active components, which determine their electron donating abilities. [24] According to result obtained the extracts of both O. corymbosa and B. pinnatum inhibit or scavenge H_2O_2 radical

The findings of present study show that the extract used is rich in phytochemicals which have significant radical scavenging activity. Further, in vitro and in vivo studies are needed to access its different activity.

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