Free radical scavenging activities of some indigenous plants of Bangladesh

Shah M. Adib1, Mohammad S. Rahman2, Mohammed Z. Rahman3, Khandaker S. Ahmed4 and Mohammad A. Rashid2*

1Department of Pharmacy, The University of Asia Pacific, Dhaka-1209, Bangladesh.
2Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh
3Centre for Biomedical Research, University of Dhaka, Dhaka-1000, Bangladesh
4Directorate of Drug Administration, Motijheel, Dhaka

Abstract:
Five indigenous plants, Bougainvillea glabra, Gmelina hystrix, Jatropha pandurifolia, Pereska grandifolia and Xylocarpus moluccensis, have been investigated for their antioxidant activity. The extracts were subjected to assay by 1,1-diphenyl-2-picrylhydrazyl (DPPH) for evaluation of free radical scavenging property. The IC50 of the organic extracts ranged from 323 to 22 μg/ml. The crude methanol extract of X. moluccensis demonstrated potent free radical scavenging activity (IC50= 22 μg/ml) as compared to tert-butyl-1-hydroxytoluene (standard) that showed an IC50 value of 91.5μg/ml. The results primarily suggest the presence of potent oxidation inhibitory principles in the bark of X. moluccensis.

Keywords: 1,1-Diphenyl-2-picryl hydrazyl, antioxidant; free radical scavenger

Introduction
There has been a worldwide positive move towards the use of traditional medicines due to the concern over the more invasive, expensive and potentially toxic mainstream modern practices (Harnett et al., 2005; WHO, 2002). Its popularity is due to the desire for more personalized health care and greater public access to health information (Greene and Peterlin, 2002).

Free radicals play a crucial role in the development of tissue damage in various human diseases such as cancer, aging, neurodegenerative disease, atherosclerosis and pathological events in living organisms (Erdemoglu et al., 2006; Gutteridge, 1994). Antioxidants may have an important role in the prevention of these diseases. There is an increasing interest in the antioxidant effects of compounds derived from plants, which could be relevant in relation to their nutritional incidence and their role in health and disease (Takahashi et al., 1992; Iwatsuki et al., 1995; Steinmetz and Potter, 1996; Wang et al., 1999; Aruoma, 1998; Bandoniene et al., 2000; Pieroni et al., 2002; Coudalid et al., 2003). A number of reports on the isolation and testing of plant derived antioxidants have been described during the past decade (Shahidi et al., 1992; Velioglu et al., 1996; Pietta et al., 1998). Extensive literature survey has revealed that no research has been conducted on the antioxidant activities of the selected plants (http://www.ncbi.nlm.nih.gov/pubmed). As a part of our effort directed to the search for such molecules from natural sources, we studied the methanol extracts of B. glabra, G. hystrix, J. pandurifolia, P. grandifolia and X. moluccensis, and we, here in, report the results of our preliminary investigation.

Materials and methods
Plant Materials: Leaves of Bougainvillea glabra (Family: Nyctaginaceae), Gmelina hystrix (Family: Lamiaceae alt. Labiatae), Jatropha pandurifolia (Family: Euphorbiaceae), Pereska grandifolia (Family: Cactaceae) and Xylocarpus moluccensis (Family: Meliaceae), were collected from Dhaka in the month of January 2007 and voucher specimens have been deposited in Bangladesh National Herbarium (BNH). The leaves the plants were first separated from the plant, cut into small pieces and air-dried for several days. The pieces were then oven dried for 24 hours at considerably low temperature to facilitate size reduction through grinding.

Extraction:
The air-dried and powdered leaves of the plants were separately extracted with methanol for 15 days at room temperature with occasional shaking and stirring. It was then filtered through a fresh cotton
plug and finally with a Whatman No.1 filter paper. The volume of the filtrate was then reduced using a Buchii Rotavapor at low temperature and pressure. Subsequent evaporation of solvents afforded extracts of B. glabra (7.3 g), J. pandurifolia (5.6 g), G. hystrix (0.8 g), P. grandifolia (0.76 g) and X. moluccensis (0.84 g). Solvent-solvent partitioning of B. glabra and J. pandurifolia was done using the protocol designed by Kupchan and modified by Van Wagenen et al. (1993) to provide n-hexane, carbon tetrachloride and chloroform soluble fractions. The crude methanol extract of G. hystrix, P. grandifolia and X. moluccensis were not subjected to partitioning due to small amount of sample.

Antioxidant activity:
The antioxidant activity (free radical scavenging activity) of the extracts on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined by the method developed by Brand-Williams et al., 1995. In the experiment, 2.0 mg of each of the extracts was dissolved in methanol. Solution of varying concentrations such as 500-, 250-, 125-, 62.50-, 31.25-, 15.62-, 7.8125-, 3.91-, 1.95 and 0.98 μg/ml were obtained by serial dilution technique. An aliquot of 2 ml of the methanol solution of the extract of each concentration was mixed with 4 ml of a DPPH-methanol solution (20 mg/L) and allowed to stand for 20 minutes for the reaction to occur. Then the absorbance was determined at 517 nm and from these values corresponding percentage of inhibitions were calculated by using the following equation:

\[ \% \text{ inhibition} = \left(1 - \frac{\text{ABS sample}}{\text{ABS control}}\right) \times 100 \]

Finally, the % inhibitions were plotted against respective concentrations used and from the graph IC₅₀ was calculated. Here, tert-butyl-1-hydroxytoluen (BHT), a potential antioxidant, was used as the positive control.

Statistics:
All the analysis was carried out in triplicate and the results are expressed as mean ± SD.

Results and discussion
The extractives of B. glabra, G. hystrix, J. pandurifolia, P. grandifolia and X. moluccensis, were assessed for free radical scavenging activity and results are presented in Table-1. 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 (Burton and Ingold, 1984; Bors et al., 1984; Mark et al., 1994). The reduction of DPPH assay has been used to detect products with antioxidant activity as free radical scavengers (Tubar et al., 1996; Cavin et al., 1998; Guse J, et al., 1998). In this study, all the extractives were shown to possess significant DPPH radical scavenging activity. X. moluccensis was found to have the highest antioxidant activity with an IC₅₀ value of 22 μg/ml. However, significant antioxidant activity was noticed by the chloroform soluble fraction of methanolic extract of J. pandurifolia (IC₅₀ 91 μg/ml), carbon tetrachloride soluble fraction of methanolic extract of B. glabra (IC₅₀ 97 μg/ml), hexane soluble fraction of methanolic extract of B. glabra (IC₅₀ 104 μg/ml) and carbon tetrachloride soluble fraction of methanolic extract of J. pandurifolia (IC₅₀ 104 μg/ml). On the other hand, moderate antioxidant activity was revealed by methanolic extract of leaves of J. pandurifolia (IC₅₀ 160 μg/ml), and its n-hexane soluble fraction (IC₅₀ 165 μg/ml) and methanolic extract of leaves of G. hystrix (IC₅₀ 170 μg/ml). The methanol extracts of B. glabra and P. grandifolia demonstrated weak free radical scavenging activity with IC₅₀ of 323 μg/ml and 450 μg/ml, respectively. Therefore, 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 activities. These plants could be subjected for extensive chromatographic separation and purification processes to isolate the bioactive compounds for the discovery of leads for further development.

Table 1: Free radical scavenging activities the test samples

<table>
<thead>
<tr>
<th>Plant</th>
<th>Samples</th>
<th>IC₅₀ (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>Green tea + Hydroxytolene</td>
<td>97.5</td>
</tr>
<tr>
<td>B. glabra</td>
<td>Hexane soluble fraction of methanolic extract</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>Carbon tetrachloride soluble fraction of methanolic extract</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>Chloroform soluble fraction of methanolic extract</td>
<td>92.3</td>
</tr>
<tr>
<td>J. pandurifolia</td>
<td>Metanolic extract of leaves</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>Hexane soluble fraction of methanolic extract</td>
<td>165</td>
</tr>
<tr>
<td></td>
<td>Carbon tetrachloride soluble fraction of methanolic extract</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>Chloroform soluble fraction of methanolic extract</td>
<td>91</td>
</tr>
<tr>
<td>G. hystrix</td>
<td>Metanolic extract of leaves</td>
<td>170</td>
</tr>
<tr>
<td>P. grandifolia</td>
<td>Metanolic extract of leaves</td>
<td>450</td>
</tr>
<tr>
<td>A. indica</td>
<td>Metanolic extract of leaves</td>
<td>22</td>
</tr>
</tbody>
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References


