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Assessment of DPPH free radical scavenging activity of some medicinal plants

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Abstract

Free radicals are one of the critical causative factors in the pathogenesis of many neurodegenerative disorders, autoimmune diseases, inflammatory conditions, diabetes, and gastrointestinal disorders. Several phytochemical and biological studies have made it evident that medicinal plants have profound antioxidant potentials that can be furnished further in the prevention and treatment of these annihilating diseases. We had assessed ethanol extracts of 08 medicinal plants from Bangladesh, for antioxidant potentials with the help of DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical scavenging assay. Those plant extracts were found to have profound antioxidant effects that could explain and justify some of their uses in traditional medicine. Among the extracts, *Ammannia multiflora, Caesalpinia pulcherrima, Dendrophthoe falcata* and *Syzygium cumini* were observed displaying strong DPPH free radical scavenging action with IC_{50} value of 6.25 µg/ml, 7.46 µg/ml, 6.22 µg/ml, 6.08 µg/ml respectively comparing to the IC_{50} value of 4.5 µg/ml of reference standard antioxidant compound ascorbic acid, with a dose reliant manner.

Key words: Free radical, Bangladesh, Antioxidant potential, DPPH (1,1,-diphenyl-2-picrylhydrazyl) assay, IC_{50} (Median Inhibitory Concentration)

Introduction

Antioxidant, a molecule which neutralizes harmful free radical compounds that damage living cells. Antioxidants can act as enzymes in the body tissue, and vitamin supplements. They are added to metals, foodstuffs, and oils routinely to prevent the damage caused by free radical.

Free radicals can be defined as atoms or groups of atoms associated with an unpaired number of electrons and can be produced when oxygen interacts with certain other molecules. These vigorously reactive free radicals, once formed can initiate a chain reaction. The most frequently encountered free radicals are the hydroxyl radical (HO•), the superoxide radical (O_2•-), the nitric oxide radical (NO•) and the lipid peroxyl radical (LOO•), while non-free radical species principally being 1 H_2O_2 and singled oxygen (O_2) (1). Nonetheless, from free radical aggression, most of all organisms are shielded by the mechanism of defenses like preventive system of antioxidant which lessens free radical formation rate, and another system is scavenging and stabilizing free radicals by producing chain-breaking antioxidants. But, when free radical production rate exceeds the capacity of the antioxidant defense mechanisms substantial tissue injury results (2). Several studies have suggested that plants are potential sources of natural antioxidant agent that play important roles in human health such as preventing oxidative damages and reduce the risks of chronic disease (3; 4). Hence, in the prevention and cure of free radical associated disorders, antioxidants with the power of free radical scavenging activities should have immense applications.

Materials and Methods

Plant material

The plant samples were collected from different parts of Bangladesh and were identified by skilled professionals in Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh.

Preparation of plant extracts

Shade drying was applied for drying whole plant (*Ecbolium linneanum*), bark (*Alstonia scholaris*), seeds (*Syzygium cumini*) and leaves (*Ammannia multiflora*), (*Caesalpinia pulcherrima*), (*Dendrophthoe falcata*), (*Xanthium indicum*) and the dried samples were grinded into coarse powder with a suitable mechanical grinder. The powder was stored in an air-tight container, and kept in a cool, dark, and dry place. The powdered plant material of each sample was extracted by cold extraction method. The filtrates (ethanol extracts) were evaporated using rotary vacuum evaporator (Bibby RE_{200}, Sterilin Ltd., UK). Then dried extracts were taken in an air-tight container, and stored in refrigerator at 4 °C to avoid any possible fungal attack.

Chemicals and Reagents

Ascorbic acid was purchased from Merck, Germany. DPPH (1,1-diphenyl-2-pycrylhydrazyl), manufactured by Sigma Chemical Co. Ltd., (St. Louis, MO, USA) was used. Solvents and all other reagents were of analytical grade.

Phytochemical Screening

The ethanol extracts of the plant(s) and plant parts (Table 01) were subjected to different preliminary phytochemical tests to identify major phytochemical groups (5; 6).

Name of Plants	Used Plant(s)/ Plant parts
Alstonia scholaris (L) R. Br.	Barks
Ammannia multiflora-Roxb.	Leaves
Caesalpinia pulcherrima Linn.	Leaves
Dendrophthoe falcata	Leaves
Ecbolium linnaenum.	Whole plant
Syzygium cumini Linn.	Seeds
Trema cannabina Lour.	Leaves
Xanthium indicum Koen.	Leaves

Table 01: Used plant(s) or plant parts of studied plants.

In Vitro Antioxidant Activity

DPPH Scavenging Assay

The stable radical DPPH has been used widely for the determination of primary anti-oxidant activity (7; 8). DPPH, after accepting electron or hydrogen radical, is converted into stable DPPH-H form. When this conversion occurs, deep violet colour of DPPH turns into light yellow colour. Unconverted DPPH is detected by UV spectrophotometer at 517 nm against blank and percent inhibition was calculated. Free radical scavenging activity of the ethanol extracts was substantiated by DPPH assay (9). Sample was prepared in ethanol at different concentrations of 500, 200, 100, 50, 10, 5, and 1 µg/mL. Sample of 1 mL of each concentration was added to 3 mL of 0.004% ethanol solution of DPPH. Incubation period of 30 min was allowed at room temperature in dark place to complete any reaction that is to be occurred. Then absorbance was measured by UV spectrophotometer at 517 nm against blank. Ascorbic acid was used as standard free radical scavenger and activity of the extract was compared with it. Activity of the sample was calculated from [(A1–A2)/A1] x100, where A1 is the absorbance of the control, and A2 is the absorbance of the standard/ sample extract. The IC_{50} values were obtained from prepared inhibition curves.

Results

Results of Phytochemical Screening

In phytochemical screening, the ethanol extracts of those said plant(s) or plant parts exhibited the presence of several groups (Table 2).

Activity in DPPH Scavenging Assay

The DPPH free radical scavenging activity of those sample extracts was in concentration dependent manner. Activity was gradually increased with the concentration at low concentration level. But, at high concentration, the graph reached plateau state. The ethanol extract of leaves of *Caesalpinia pulcherrima* showed highest IC_{50} value of 7.46 µg/mL, while standard ascorbic acid showed IC_{50} value of 4.5 µg/mL. Again, the ethanol extract of leaves of *Xanthium indicum* showed lowest IC_{50} value of 90.43 µg/mL.

see Figure 1.

Discussion

As, the lower the IC_{50}, the higher the antioxidant activity of the extracts (10), DPPH scavenging assay was carried out to determine the IC_{50} of each plant extract, for evaluating *in vitro* antioxidant activity. Phytochemical investigation revealed the presence of flavonoids, phenolic compounds, and tannins in some of the plant extracts, and it is well established that flavonoids are responsible for antioxidant properties (11; 12). Phenolic compounds and flavonoids have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals (13; 14). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (15).Tannins which were presented in some of plant extracts had been observed to have remarkable activity in cancer prevention and anticancer (16). In carcinogenesis, reactive oxygen species are responsible for initiating the multistage carcinogenesis process starting with DNA damage and accumulation of genetic events in one or few cell lines which leads to progressively dysplastic cellular appearance, deregulated cell growth, and finally carcinoma (17).

Over production of free radicals lead to oxidative stress. Oxidative damage of proteins, DNA and lipid is associated with chronic degenerative diseases including cancer, coronary artery disease, hypertension, diabetes etc (18). And, compounds that can scavenge free radicals have great potential in ameliorating these disease processes (19; 20; 21). Consequently, therapy using free-radical scavenging antioxidants has potential to prevent, delay or

ameliorate many of these disorders (22). And, as some of the plant extracts exhibited concentration dependent DPPH radical scavenging activities which were strongly comparable to the standard antioxidant ascorbic acid, they can be used after furnishing properly to treat these types of disorders.

Conclusion

The present results recommend that among the tested plant extracts, *Ammannia multiflora, Caesalpinia pulcherrima, Dendrophthoe falcata* and *Syzygium cumini* have potent antioxidant activities. A number of constituents are identified in the extracts studied and makes it difficult to credit the antioxidant properties accordingly to any single constituent's group without advance studies which are beyond the extent of this paper. Thus, advance extensive studies are quite necessary to uncover these plant(s) or plant parts active antioxidative principles.

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References

1. Yildirim A, Mavi A, Oktay M, Kara AA, Algur OF, Bilaloglu V. Comparison of antioxidant and antimicrobial activities of tilia (Tilia argenta DesfEx DC), sage (Salvia triloba L.) and black tea (Camellia sinensis) extracts. J. Agri. Food Chem 2000; 48: 5030-5034.
2. Rahman MAA, Moon SS. Antioxidant polyphenol glycosides from the Plant Draba nemorosa. Bull. Korean Chem. Soc 2007; 28(5): 827-831.
3. Demiray S, Pintado ME, Castro PML. Evaluation of phenolic profiles and antioxidant activities of Turkish medicinal plants: Tilia argentea, Crataegi folium leaves and Polygonum bistorta roots. World Academy of Science, Engineering and Technology 2009; 54: 312-317.
4. Dimitrios B. Sources of natural phenolic antioxidants. Trends Food Sci. Tech 2006; 17: 505-512.
5. Kokate CK. Practical Pharmacognosy. 4th ed. Delhi: Vallaph Prakashan, 1994; p. 107-111.
6. Khandelwal KR. Practical Pharmacognosy. 11th ed. Pune: Nirali Prakashan, 2004; p. 149-153 & 157-159.
7. Brand-Williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. Lebanon Wissen Technology 1995; 28: 25-30.
8. Katalinic V, Milos M, Modun D, Music I, Boban M. Antioxidant effectiveness of selected wines in comparison with (+)-calectin. Food Chem 2004; 86: 593-600.
9. Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Eslami B. Free radical scavenging ability of methanolic extract of Hyoscyamus squarrosus leaves. Pharmacologyonline 2009; 2: 796-802.
10. Maisuthisakul P, Suttajit M, Pongsawatmanit R. Assessment of phenolic content and free radicalscavenging capacity of some Thai indigenous plants. Food Chem 2007; 100: 1409-1418.
11. Beecher GR. Overview of dietary flavonoids: nomenclature, occurrence and intake. J Nutr 2003; 133: 3248S-3254S.
12. Ortega R. Importance of functional foods in the Mediterranean diet. Public Health Nutr 2006; 9: 1136-1140.
13. Rice-Evans C, Sampson J, Bramley PM and Holloway DE. Why do we expect carotenoids to be antioxidants in vivo. Free Radical Research 1997; 26: 381–398.
14. Jorgensen LV, Madsen HL, Thomsen MK, Dragsted LO and Skibsted LH. Regulation of phenolic antioxidants from phenoxyl radicals: An ESR and electrochemical study of antioxidant hierarchy. Free Radical Research 1999; 30: 207-220.
15. Hasan SM, Hossain, MM, Faruque A, Mazumder, MEH, Rana MS, Akter R, Alam MA. Comparison of antioxidant potential of different fractions of Commelina benghalensis Linn. Bang. J. Life. Sci 2008; 20 (2): 9-16.
16. Li H, Wang Z, liu Y. Review in the studies on tannins activity of cancer prevention and anticance. Zhong-Yao-Cai 2003; 26(6): 444-448.
17. Tsao AS, Kim ES, Hong WK. Chemoprevention of Cancer. CA Cancer J. Clin 2004; 54: 150-180.
18. Lee KG, Mitchell AE, Shibamoto T. Determination of antioxidant properties of aroma extracts from various beans. J. Agric. Food Chem 2000; 48: 4817-4820.
19. Kris-Etherton PM, Hecker KD, Bonanome A, Cival SM, Binkasi AE, Hilpert KF. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am. J. Med 2002; 113: 71s-88s.
20. Di Malteo V, Esposito E. Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimers disease, Parkinson disease and amyotrophic lateral sclerosis. Current Drug Target- CNS and Neurological Disorders 2003; 2: 95-107.
21. Behera BC, Verma N, Sonone A, Makhaija U. Determination of antioxidant potential of lichen Usnea ghattensis in vitro. LWT Food Science and Technology 2006; 39: 80-85.
22. Delanty N, Dichter MA. Antioxidant therapy in neurologic diseases. Arch. Neurol 2000; 57(9): 1265-1270.

Name of Plants	Results of Phytochemical Groups							
	Alkaloids	Flavonoids	Glycosides	Phenolic group	Reducing sugars	Saponins	Steroids	Tannins
Alstonia scholaris (L) R. Br.	+	+	-	+	+	+	+	+
Ammannia multiflora-Roxb.	+	-	+	+	+	-	-	+
Caesalpinia pulcherrima Linn.	+	+	+	+	-	-	+	+
Dendrophthoe falcata Linn.	+	-	+	+	+	-	-	+
Ecbolium linneanum Kurz.	+	+	+	-	-	+	-	-
Syzygium cumini Linn.	+	+	+	+	+	-	-	+
Trema cannabina Lour.	+	-	-	-	+	-	+	+
Xanthium indicum Koen.	+	+	+	-	+	-	-	+

Table 02: Phytochemical screening of studied plant(s) or plant parts.

+ = Presence - = Absence

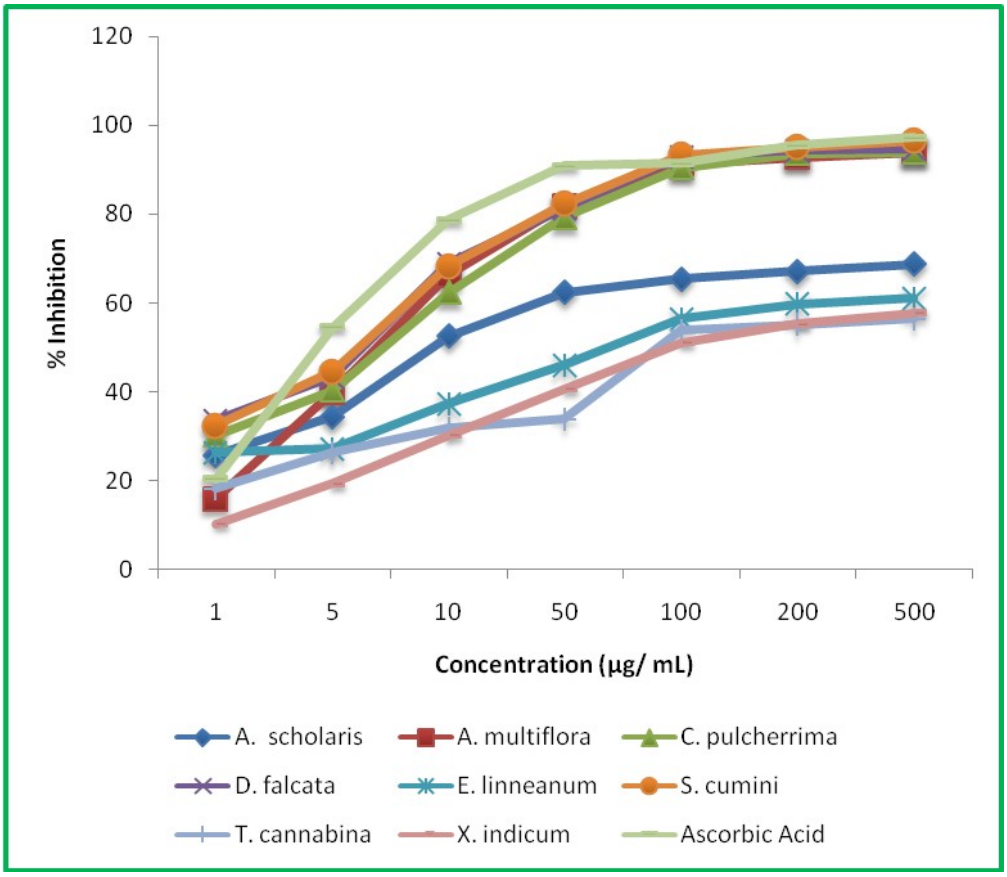

Figure 1: DPPH scavenging activity of studied plant(s) or plant parts