

Full Length Research Paper

DPPH free radical scavenging activity of some Bangladeshi medicinal plants

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Hydromethanol extracts of 15 Bangladeshi medicinal plants, traditionally used in different ailments, were evaluated for antioxidant potential using DPPH (1,1-diphenyl-2-picryl hydrazyl) radical scavenging assay. Among the extracts *Cocos nucifera*, *Caesalpinia pulcherrima*, *Punica granatum* and *Syzygium cumini* were found displaying strong (90% or more) DPPH radical scavenging action. *Syzygium cumini* exhibited the highest radical scavenging, with an IC₅₀ value of 4.25 µg/ml compared to the IC₅₀ value of 5.15 µg/ml as shown by the reference antioxidant ascorbic acid, in a dose dependent fashion.

Key words: Antioxidant, medicinal plants, Bangladesh, DPPH free radical.

INTRODUCTION

Oxygen is, no doubt, an indispensable part of aerobic life. However, under certain circumstances, it can seriously affect our well being through the formation of reactive oxygen species (ROS) representing both free radical and non-free radical species, and their potential deleterious effects such as atherosclerosis, ischaemic heart disease, ageing, inflammation, diabetes, immunosuppression, neurodegenerative diseases, cancer and others (Jadhav and Bhutani, 2002; Gulcin et al., 2002).

The most frequently encountered free radicals are the hydroxyl radical (HO•), the superoxide radical (O₂•-), the nitric oxide radical (NO•) and the lipid peroxy radical (LOO•) while non-free radical species principally being H₂O₂ and singlet oxygen (¹O₂) (Yildirim et al., 2000). Nevertheless, almost all organisms are protected from free radical attack by defense mechanisms such as a preventive antioxidant system that reduces the rate of free radical formation, and another is a system to produce chain-breaking antioxidants that scavenge and stabilize free radicals. But, when free radical production rate exceeds the capacity of the antioxidant defense

mechanisms substantial tissue injury results (Rahman and Moon, 2007). Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and therapeutics of free radical mediated diseases.

Again, Bangladesh is blessed with rich floristic and faunastic resources with particular reference to the antioxidant components from medicinal plants. So well designed, systematic and objective research in this area will benefit our people who are happened to be plagued with various ailments, and lack technological and economic resources to cope up with them with orthodox medicine. Considering the importance of this area and as a part of our ongoing investigation on natural antioxidants from local medicinal plants of Bangladesh (Hasan et al., 2008), in this paper, we reported a study of the antioxidant activity of 15 Bangladeshi medicinal plants, which are traditionally used in folkloric remedies for various disorders where free radicals are thought to be involved.

MATERIALS AND METHODS

Chemicals

DPPH (1, 1-diphenyl, 2-picrylhydrazyl) was obtained from Sigma Chemical Co. USA. Ascorbic acid was obtained from SD Fine

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Table 1. DPPH radical scavenging activities of some Bangladeshi medicinal plants.

Plant Name	Family	Part (s) used	Accession No.	IC ₅₀ (µg/ml)
<i>Artocarpus lacucha</i> Buch.-Ham.	Moraceae	Leaves, Fruit pericarp.	32614	54.74 (Leaves) 39.93 (Pericarp)
<i>Baccaurea ramiflora</i> Lour.	Phyllanthaceae	Fruit pericarp	*	31.38
<i>Butea monosperma</i> (Lam.) Taub.	Papilionaceae	Leaves	32616	25.96
<i>Caesalpinia pulcherrima</i> Linn.	Ceasalpiniaceae	Leaves	32782	16.0
<i>Cocos nucifera</i> Linn.	Arecaceae	Developing kernel	*	13.67
<i>Commelina benghalensis</i> Linn.	Commelinaceae	Aerial parts	32784	21.53
<i>Curcuma alismatifolia</i> Gangnep.	Zingiberaceae	Leaves	32787	18.72
<i>Feronia limolia</i> Linn.	Rutaceae	Leaves	*	17.60
<i>Hopea odorata</i> Roxb.	Dipterocarpaceae	Leaves	32613	33.03
<i>Ipomoea quamoclit</i> Linn.	Convolvulaceae	Aerial parts	*	25.96
<i>Michelia champaca</i> Linn.	Magnoliaceae	Leaves	32615	22.43
<i>Punica granatum</i> Linn.	Punicaceae	Fruit peel	32895	10.82
<i>Syzygium cumini</i> Linn.	Myrtaceae	Seeds	32893	4.25
<i>Tinospora cordifolia</i> (Wild.) Miers.	Menispermaceae	Aerial parts	*	29.87
<i>Xanthium indicum</i> Koenig.	Asteraceae	Leaves	32785	23.44

[†]Identified by Professor Dr. Abdul Ghani, Department of Pharmacy, Stamford University Bangladesh.

Chem. Ltd., Biosar, India.

Plant material

The plant materials (Table 1) were collected between February and April 2008 from different regions of Bangladesh. Most of the samples were identified by experts in Bangladesh National Herbarium, Mirpur, Dhaka and the rest by Professor Dr. Abdul Ghani (Professor of Pharmacognosy, Stamford University Bangladesh). The specimen samples are kept in the Bangladesh National Herbarium and Laboratory of Pharmacognosy in the Department of Pharmacy, Stamford University Bangladesh.

DPPH radical scavenging activity

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined by the method described by Braca et al. (2001). Plant extract (0.1 ml) was added to 3ml of a 0.004% methanol solution of DPPH. Absorbance at 517nm was determined after 30 min, and the percentage inhibition activity was calculated from $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/ standard. The inhibition curves were prepared and IC₅₀ values were obtained.

RESULTS AND DISCUSSION

DPPH test, which is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action. Ascorbic acid was chosen as the reference antioxidant for this test. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517 nm and also for a visible deep

purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. The IC₅₀ values of all the 15 different plant extracts have been furnished in the Table 1. Highest scavenging was observed with *Syzygium cuminii* seed extract with an IC₅₀ value of 4.25 µg/ml as opposed to the IC₅₀ value of ascorbic acid 5.14 µg/ml, which is a well known antioxidant (Figure 1). Scavenging of DPPH radical was found to rise with increasing concentration of the extracts. (Table 2)

Additionally, it has been determined that the antioxidant effect of plant products is mainly due to radical-scavenging activity of phenolic compounds such as flavonoids, polyphenols, tannins, and phenolic terpenes (Rahman and Moon, 2007). 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 (Hasan et al., 2008). Oxidative injury now appears the fundamental mechanism underlying a number of human neurologic and other disorders such as inflammation, viral infections, autoimmune pathologies, and digestive system disorders including gastrointestinal inflammation and ulcer (Aruoma, 2003). For instance in diabetes, increased oxidative stress which co-exist with reduction in the antioxidant status has been postulated: Oxygen free-radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes, and play a role in the long

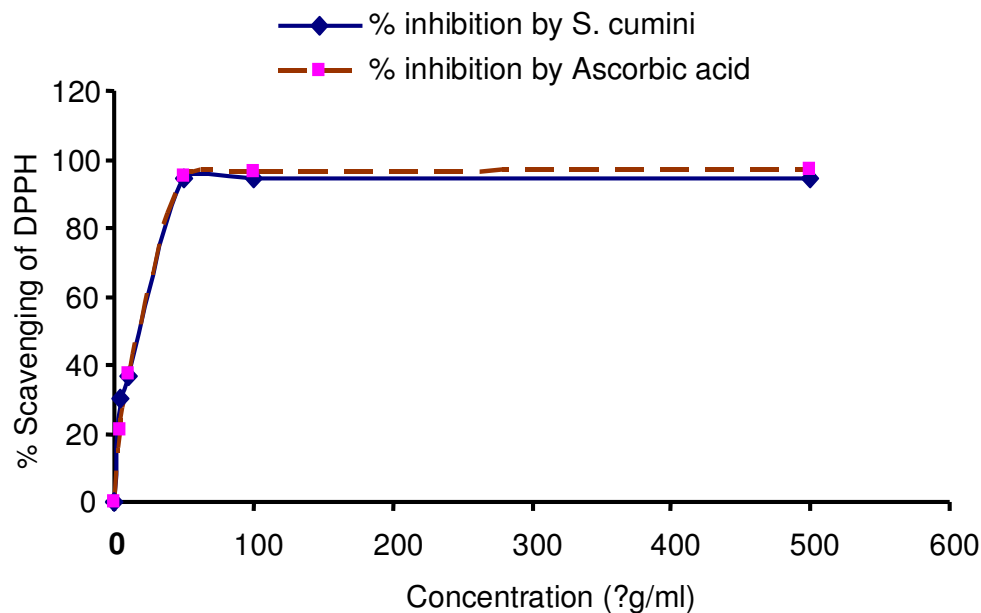


Figure 1. DPPH radical scavenging activity of the hydromethanol extracts *Syzygium cumini*. Values are the average of duplicate experiments and represented as mean \pm SD.

term complication of diabetes (Sabu and Kuttan, 2002; Atawodi, 2005). Similarly, in carcinogenesis, reactive oxygen species are responsible for initiating the multi-stage 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 (Tsao et al., 2004). Hence, therapy using free-radical scavenging antioxidants has potential to prevent, delay or ameliorate many of these disorders (Delanty and Dichter, 2000). Over the past two decades, an expanding body of evidence from epidemiological and laboratory studies have demonstrated that some edible plants as a whole, or their identified ingredients with antioxidant properties have substantial protective effects on human carcinogenesis (Greenwald, 2002; Tsao et al., 2004; Kinghorn et al., 2004; Mehta and Pezzuto, 2002).

The present results suggest that all the tested plant extracts have moderate to potent antioxidant activity. Since a variety of constituents are known from the extracts studied, it becomes difficult to ascribe the antioxidant properties selectively to any one group of constituents without further studies which are beyond the scope of this paper. Thus, further extensive investigations are necessary to find out the active antioxidative principles present in these plants.

REFERENCES

Akter R, Hasan SMR, Siddiqua SA, majumder MM, Hossain MM, Alam MA, Haque S, Ghani A (2008). Evaluation of analgesic and antioxidant potential of the leaves of *Curcuma alismatifolia* gagnep. S. J.

- Pharm. Sci. 1 (1 &2): 3-9.
- Aruoma OI (2003). Methodological considerations for characterizing potential antioxidant actions of bioactive components in food plants. *Mutat. Res.* 523 – 524:9-20.
- Atawodi SE (2005). Antioxidant potential of African medicinal plants. *Af. J. Biotech.* 4 (2): 128-133.
- Ávila-Peña D, Peña N, Quintero L, Suárez-Roca H (2007). Antinociceptive activity of *Syzygium jambos* leaves extract on rats. *J. Ethnopharmacol.* 112 (2): 380-385.
- Banerjee A, Dasgupta N, and Bratati De (2005). *In vitro* study of antioxidant activity of *Syzygium cumini* fruit. *Food Chem.* 90 (4): 727-733.
- Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I (2001). Antioxidant principles from *Bauhinia terapotensis*. *J. Nat. Prod.* 64: 892-895.
- Delanty N, Dichter MA (2000). Antioxidant therapy in neurologic diseases. *Arch. Neurol.* 57(9):1265-1270.
- Ghani A (2003). Medicinal Plants of Bangladesh-Chemical constituents and uses, 2nd edition, The Asiatic Society of Bangladesh., Dhaka.
- Greenwald P (2002). Science, Medicine and the future of Cancer Chemoprevention. *Br. Med. J.* 324: 714-718.
- Gulcin I, Buyukokuroglu ME, Oktay M, Kufrevioglu OI. (2002). On the *in vitro* antioxidant properties of melatonin. *J. Pineal Res.* 33:167-171.
- Hasan SM, Hossain, MM, Faruque A, Mazumder, MEH, Rana MS, Akter R, Alam MA (2008). Comparison of antioxidant potential of different fractions of *Commelina benghalensis* Linn. *Bang. J. Life. Sci.* 20 (2): 9-16.
- Jadhav HR, Bhutani. KK (2002). Antioxidant properties of Indian medicinal Plants. *Phytother. Res.* 16: 771-773.
- Kinghorn AD, Su BN, Jang DS, Chang LC, Gu JQ, Carcache-Blanco EJ, Pawlus AD, Lee SK, Park EJ, Cuendet M, Gills JJ, Bhat, HS, Meta Greenwood E, Song LL, Jang M, Pezzuto JM (2004). Natural inhibitors of carcinogenesis. *Planta Med.* 70(8): 691-705.
- Likhitwitayawuid K, Somsute A, Sritularak B, Polypradith P (2006). Chemical transformations of oxyresveratrol (trans-2,4,3',5',-tetrahydroxy stilbene) into a potent tyrosinase inhibitor and a strong cytotoxic agent. *Bioorg. Med. Chem. Lett.* 16(1): 5650-5653.
- Mehta RG, Pezzuto JM. (2002). Discovery of cancer preventive agents from natural products: from plants to prevention. *Curr. Oncol. Rep.* 4(6):478-486.
- Mongolsuk S, Robertson A, and Towers R. (1957). 2,4,3',5'- Tetrahydro-

Table 2. Traditional uses phytochemical constituents and reported bioactivity of the studied plants.

Plant Name	Traditional uses	Constituents	Reported bioactivity	References
<i>Artocarpus lacucha</i> Buch.-Ham.	Applied to pimples, cracked skin and sores.	Triterpines, lupeol, glycoflavanol, tannin.	Anthelmintic, antiherpetic, antimycobacterial, anticancer.	Likhiwitayawuid, K. et al., 2006, Puntumchai et al., 2004, Mongolsuk et al., 1957, Singh et al., 2001
<i>Baccaurea ramiflora</i> Lour.	Young leaves as vegetables, flavoring agent with curries and minced meat.	6'-O-vanilloylisotachioside (1) and 6'-O-vanilloyltachioside.	Antioxidant.	Yang et al. 2007.
<i>Butea monosperma</i> (Lam.) Taub.	As astringent; in gout and skin diseases; as aphrodisiac, expectorant, tonic, diuretic, antipyretic.	Triterpene, flavonoids, tannins, oleic and linoleic acid, palmitic acid, kino-tannic acid, gallic acid, lupenone, lupeol.	Antioxidant, antistress, hepatoprotective, anthelmintic, antidiarrhoeal, anti-diabetic, anti-inflammatory	Sharma and Garg 2009
<i>Caesalpinia pulcherrima</i> Linn.	Liver disorder, cough, bronchitis, asthma, chronic catarrh, malarial fever, intestinal worms, cholera, dysentery, certain skin diseases.	Gallic acid, flavonoids, lupeol, quercetin, rutin, tannins, lectins.	Anticancer, astringent.	Ghani, 2003.
<i>Cocos nucifera</i> Linn.	Rehydrating agent in cholera, diarrhea and dysentery; treatment of cancer; as a hair nutrient in alopecia.	Fixed oil rich in tocopherol, fatty alcohol, triterpene alcohol, sterols, gum.	Diuretic, astringent, antibiotic, antiseptic, antifungal.	Ghani, 2003.
<i>Commelina benghalensis</i> Linn.	Otitis media, suppurative sores, snakebite, swelling, burns.	Anthocyanins, dammarane triterpene, sterols, campesterol.	Demulcent, emollient, refrigerant, laxative, hypotensive, CNS depressant, diuretic.	Ghani, 2003.
<i>Curcuma alismatifolia</i> Gangnep.	Applied to bruises and sprains; skin diseases, inflammatory disorders	Alkaloid, flavonoid, gum.	Analgesic, antioxidant.	Akter et al., 2008
<i>Feronia limolia</i> Linn.	As remedy for bites of venomous insects and reptiles, heart diseases.	Citric acid, stigmasterol, alkaloids, coumarins.	Astringent, tonic, antidiarrhoeal, aphrodisiac, antitumour.	Ghani, 2003.
<i>Hopea odorata</i> Roxb.	Gingivitis, as an ointment for sores and wounds.	Resin, beta sitosterol, hopeaphenol.	Antibacterial, cytotoxic.	Ghani, 2003.
<i>Ipomoea quamoclit</i> Linn.	Applied to carbuncles and bleeding piles; in snakebite; as purgative.	Alkaloids, cyanogenetic glycosides, quamoclins I-IV, jalapin.	Treatment of ulcer and breast pain	Ghani, 2003.
<i>Michelia champaca</i> Linn.	Expectorant, stimulant, astringent, febrifuge, diuretic, anti-inflammatory	sesquiterpene lactones, essential oils, alkaloids, bet-sitosterol, tertiary monophenolic base	Leishmanicidal, anti-inflammatory, antipyretic, hepatoprotective, Antimicrobial	Vimala et al., 1997.
<i>Punica granatum</i> Linn.	Refrigerant, stomachic.	Tannin, citric acid, carotenoids, anthocyanins, ascorbic acid, thiamine, riboflavine.	Astringent, antidiarrhoeal, anthelmintic, antibacterial, antiviral.	Ghani, 2003.

Table 2. Contd

<i>Syzygium cumini</i> Linn.	As gargle and mouthwash; in diarrhea, dysentery, stress; as nourishing, stomachic, carminative.	Gallic acid, ellagic acid, triterpenoids, flavonoids, quercetin, Vit-A, C, B1, B2.	Antioxidant, antidiabetic, antibacterial, antinociceptive	(Ghani, 2003), (Banerjee et al.,2005), (Rao and Rao, 2001), (Ávila-Peña et al., 2007)
<i>Tinospora cordifolia</i> (Wild.) Miers.	General tonic, antiperiodic, anti-spasmodic, anti-inflammatory, antiarthritic, anti-allergic and anti-diabetic.	Alkaloids, Diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics.	Neroprotective, cardioprotective, antioxidant, anti-diabetic, anti-inflammatory, anti-arthritic, anti-stress, hepatoprotective, and anti-neoplastic	Desai et al (2002), Singh et al (2003).
<i>Xanthium indicum</i> Koenig.	Urinary complaints, sores of lips and mouth, inflammatory swellings.	Tocopherols, plastoquinone, polyphenols.	Antimalarial, trypanocidal, anticancer.	Ghani, 2003.

xystilbene from *Artocarpus lakoocha*. J. Chem. Soc. pp. 2231-2233.

Puntumchai A, Kittakoop P, Rajviroongit S, Vimuttipong S, Likhitwitayawuid K, Thebtaranonth (2004). Lakoochins A and B, new antimycobacterial stilbene derivatives from *Artocarpus lakoocha*. Y. J Nat. Prod. 67(3): 485-486.

Rao BK, Rao CA (2001). Hypoglycemic and antihyperglycemic activity of *Syzygium alternifolium* (Wt.) Walp. seed extracts in normal and diabetic rats. Phytomedicine 8(2): 88-93.

Rahman MAA, Moon SS (2007).Antioxidant polyphenol glycosides from the Plant *Draba nemorosa*. Bull. Korean Chem. Soc. 28(5): 827-831.

Sabu MC, Kuttan R (2002). Antidiabetic activity of medicinal plants and its relationship with their antioxidant property. J. Ethnopharmacol. 81: 155-160.

Sharma N, Garg V (2009). Antidiabetic and antioxidant potential of ethanolic extract of *Butea monosperma* leaves in alloxan-induced diabetic mice. Ind. J. Biochem. Biophys. 46: 99-105.

Singh DB, Attri BL, Sharma TVRS, Sreekumar PV (2001). Nutrient composition of some wild edible fruits of Andaman and Nicobar Islands. J. Appl. Hortic. 3(1): 60-62.

Tsao AS, Kim ES, Hong WK (2004). Chemoprevention of Cancer. CA Cancer J. Clin. 54: 150-180.

Vimala R, Nagarajan S, Alam M, Susan T, Joy S (1997). Antiinflammatory and antipyretic activity of *Michelia champaca* Linn., (white variety), *Ixora brachiata* Roxb. and *Rhynchosia cana* (Willd.) D.C. flower extract. Indian. J. Exp. Biol. 35(12):1310-1314.

Yildirim A, Mavi A, Oktay M, Kara AA, Algur OF, Bilaloglu V (2000). 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. 48: 5030-5034.

Yang XW, Wang JS, Ma YL, Xiao HT, Zuo YQ, Lin H, He HP, Li L, Hao XJ (2007) Bioactive phenols from the leaves of *Baccaurea ramiflora*. Planta Med. 73(13):1415-141