SCREENING OF DIFFERENT PARTS OF THE PLANT *PANDANUS ODORUS* FOR ITS ANTIOXIDANT ACTIVITY

Kaiser Hamid[1]* Monika Rani Saha[2] Kaniz Fatima Urmi[3] Md. Razibul Habib [4]
Muhammad Mukhlesur Rahman[5]

*[1]Department of Pharmacy, East West University, Mohakhali, Dhaka-1212
[2]Department of Pharmacy, Noakhali Science and Technology University
[3]Department of Pharmacy, Jahangirnagar University
[4]Department of Pharmacy, International Islamic University Chittagong
[5]Department of Pharmacy, Southeast University

ABSTRACT: The present study was undertaken to explore the antioxidant potential of different parts of the plant *Pandanus odorus* that is believed to be useful in the treatment of diabetes.1,1-diphenyl-2-picrylhydrazyl (DPPH) was used in determining the free radical scavenging activity and ascorbic acid was used as standard. All the fractions of both leaves and root showed potential antioxidant activity. In case of leaves the chloroform fraction showed highest radical scavenging activity with IC_{50} value of 1.66 µg/ ml followed by petroleum ether and ethyl acetate fraction having IC_{50} value of 4.69 µg/ ml and 26.21 µg/ ml respectively. In case of root, the petroleum ether fraction showed highest radical scavenging activity with IC_{50} value of 1.42µg/ ml followed by ethyl acetate and chloroform fraction having IC_{50} value of 3.76 µg/ ml and 7.01 µg/ ml respectively.

Key words: DPPH, free radical, *Pandanus odorus,* chloroform, petroleum ether, ethyl acetate.

INTRODUCTION

The free radicals species contain unpaired electrons. The oxygen radicals, icluding superoxide radical (O^{2-}), hydroxyl radical (OH^{-}) and non-free radical species, such as H_2O_2 and singlet oxygen ($_1O^2$), are various forms of activated oxygen (Gulcin *et al*., 2002; Yildirim *et al.,* 2000), generated in many redox processes. These radicals are trapped and destroyed by specific enzymes, such as superoxide dismutase, catalase and glutathione peroxidase. Overproduction of free radicals, together with A, C and E avitaminosis and a reduced level of the above mentioned enzymes, is considered to be the main contributor to oxidative stress (Ellnain-Wojtaszek *et al*., 2003).

Besides, excessive generation of ROS, induced by various stimuli and which exceed the antioxidant capacity of the organism, leads to a variety of pathophysiological processes such as inflammation, diabetes, genotoxicity, and cancer (Kourounakis *et al*., 1999, Gulcin *et al*., 2002, Gulcin *et al.,* 2003).

Pandanus odorus Ridl. (Fragrant screw pine, Thai name: Toei-hom) belongs to the family Pandanaceae is an upright shrub, approximately 0.5–1.0 m high, consisting of stem and nominal support roots. Various parts of Toei-hom are used in food and traditional medicine. Patients and medical practitioners believe that the root and rhizome to be effective against diabetes (Phongboonrod, 1976; Ketusing, 1988). The decoction of the *P. odorus* root and rhizome has been traditionally used in treating diabetic patients without much scientific evidence.

It was reported that the root and rhizome of *Pandanus odorus* exert both hypoglycemic and hypolipidemic activity. At the same time isolation of 4- hydroxybenzoic acid from the root of *P. odorus* having hypoglycemic activity has been confirmed by Penchom *et al,* 1998.
As a part of our continuous effort to explore the antioxidant potential of the plants of Bangladesh, the aim of this research is to analyze the antioxidant activity of different parts of the plant *P. odorus.*

MATERIALS AND METHODS

Plant materials:

Different parts of the test plants were collected during the month of January, 2010 from Ramnagar, Comilla, Bangladesh and identified from the Bangladesh National Herbarium, Dhaka where a voucher specimen was deposited having the accession no. 34478

Preparation of Crude Plant Extract:

About 200 g of dried, ground separate parts of the plant were soaked in 1.5 L of 98% methanol for 5-7 days, stirring every 18 h using a sterilized glass rod, separately. The final extracts were passed through No. 1 Whatman filter paper (Whatman Ltd., UK) that is followed by solvent-solvent partitioning with petroleum ether, chloroform and ethyl acetate (Haque *et al.,* 2008). The filtrates obtained were concentrated under vacuum in a rotary evaporator at 40 °C and stored at 4°C for further use.

Screening of Antioxidant Activity:

Antioxidant activities of different parts of the test plant were determined on the basis of their scavenging potential of the stable DPPH free radical in both qualitative and quantitative assay.

Qualitative assay:

A suitably diluted stock solutions were spotted on pre-coated silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to resolve polar and non-polar components of the extracts. The plates were dried at room temperature and were sprayed with 0.02% DPPH in ethanol. Bleaching of DPPH by the resolved band was observed for 10 minutes and the color changes (yellow on purple background) were noted (Sadhu *et al.*, 2003).

Quantitative assay:

The antioxidant activity of different parts of the plants *Pandanus odorus* were determined using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay (Hasan *et. al*, 2006; Koleva *et. al,* 2002; Lee *et. al,* 2003) . The free radical scavenging capacity of the petroleum ether, chloroform and ethyl acetate fractionated extracts of different parts of the plant were determined using DPPH. DPPH solution (0.004% w/v) was prepared in 95% methanol. The crude extracts of different parts of the plant *Pandanus odorus* were mixed with 95% methanol to prepare the stock solution (10mg/100mL). The concentration of extract of different parts of *Pandanus odorus* solution was 10 mg /100 ml or 100µg/ml. From stock solution 2 ml, 4 ml, 6 ml, 8 ml & 10 ml of this solution were taken in five test tubes & by serial dilution with methanol and was made the final volume of each test tube up to 10 ml whose concentration was then 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml & 100µg/ml respectively. Freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes containing extract of different parts of *Pandanus odorus* (20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, and 100µg/ml) and after 10 min, the absorbance was taken at 517 nm using a spectrophotometer.

Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration (10mg/100mL or 100µg/ml) of extract of different parts of *P.odorus*. Control sample was prepared containing the same volume without any extract and reference ascorbic acid. 95% methanol was used as blank. Percent scavenging of the DPPH free radical was measured using the following equation-

% DPPH radical-scavenging = [(Absorbance of Control - Absorbance of test Sample) / (Absorbance of Control)] x 100

Then % inhibitions were plotted against respective concentrations used and from the graph (Fig I and Fig II) IC_{50} was calculated.

RESULTS

In case of leaves, the chloroform fraction showed highest radical scavenging activity having IC_{50} value of 1.66 µg/ ml followed by petroleum ether and ethyl acetate fraction having IC_{50} value of 4.69 µg/ ml and 26.21 µg/ ml respectively. In case of root, the petroleum ether fraction showed highest radical scavenging activity having IC_{50} value of 1.42 µg/ ml followed by ethyl acetate and chloroform fraction having IC_{50} value of 3.76 µg/ ml and 7.01 µg/ ml respectively. (Fig I and Fig II). On the other hand the antioxidant activity of standard (Ascorbic Acid) was with IC_{50} value of 43.04µg/ml.

Fig I: IC_{50} values of different fractionated extract of leaves of *P. odorus*

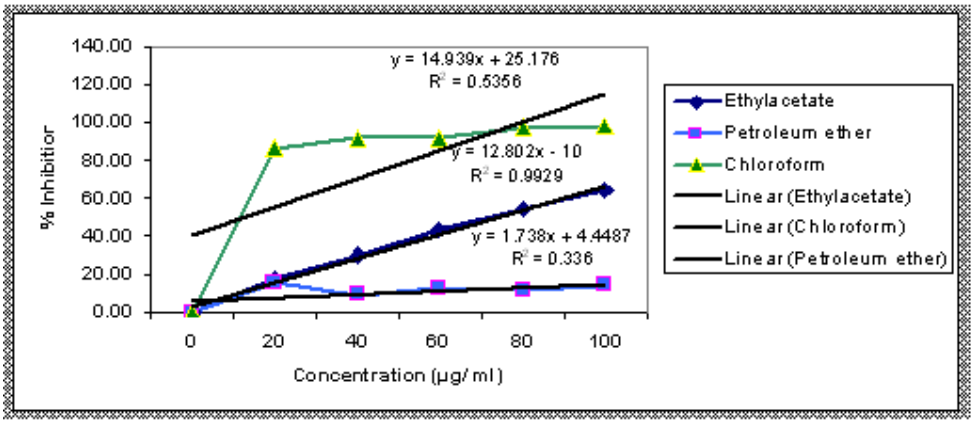
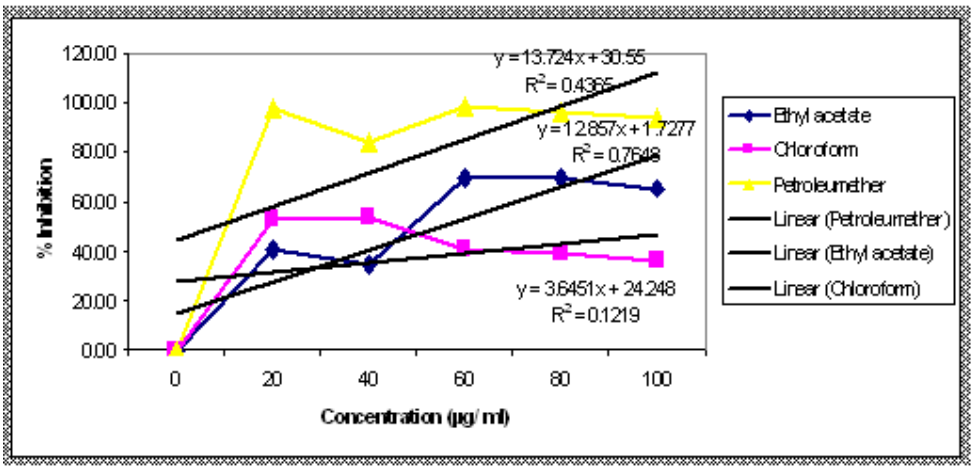


Fig II: IC_{50} values of different fractionated extract of root of *P.odorus*



DISCUSSION:

Antioxidant properties, particularly radical scavenging activities, are very important due to the harmful role of free radicals in foods and biological systems. The reduction of DPPH absorption is indicative of the capacity of the extracts to scavenge free radicals, independently of any enzymatic activity. The method widely used to predict the ability of flavonoids to transfer H atoms to radicals is based on the free radical, 1, 1-diphenyl-2-picrylhydrazyl in the DPPH assay. (Hung-Ju Chou *et al*., 2009)

It is obvious that the constituents like tannins, reducing sugars and proteins present in the extract may be responsible for such activity. (Nooman A. *et al.,* 2005)

The antioxidant potential of the extracts studied here could be attributed to flavanoids. The key role of phenolic compounds as scavengers of free radicals is emphasized in several reports (Madsen *et al*., 1996; Moller *et al*., 1999). Polyphenolic compounds are known to have antioxidant activity and it is likely that the activity of the extracts may be due to these compounds (Okudu *et al.,* 1996, Tepe *et al*., 2006). This activity is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Zheng *et al*., 2001). In fact, many medicinal plants contain large amounts of antioxidants such as polyphenols. Many of these phytochemicals possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases (Anderson *et al.,* 2001, Djeridane *et al.,* 2006).

Acknowledgement:

The authors are thankful to Ms. Mahmuda Haque, Acting Chairperson, Department of Pharmacy, Southeast University, Dhaka-1213 for her support during the project work.

REFERENCES

Anderson K J, Teuber S S, Gobeille A, Cremin P, Waterhouse A L, Steinberg F M (2001). Walnut polyphenolics inhibit in vitro human plasma and LDL oxidation. Biochemical and molecular action of nutrients. *J. Nutr.* 131, 2837-2842.

Chou H J, Kuo J T, En-Shyhlin, (2009). Comparative Antioxidant Properties of Water Extracts from Different Parts of Beefsteak Plant (Perilla frutescens), *Journal of Food and Drug Analysis*, 17 (6): 489-496

Djeridane A, Yousfi M , Nadjemi B, Boutassouma D, Stocker P, Vidal N (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem.* 97, 654-660.

Ellnain-Wojtaszek M, Kruczynski Z, Kasprzak J, (2003). Investigation of the free radical scavenging activity of Ginkgo biloba L. leaves. *Fitoterapia,* 74, 1–6.

Gulcin I, Buyukokuroglu ME, Oktay M, Kufrevioglu IO, (2002).On the in vitro antioxidant properties of melatonin. *J. Pineal Res.* 33, 167-171.

Gulcin I, Buyukokuroglu M E, Oktay M, Kufrevioglu I O, (2003). Antioxidant and analgesic activities of turpentine of *Pinus nigra Arn.* Subsp. Pallsiana (Lamb.) Holmboe. *Journal of Ethnopharmacology,* 86, 51-58.

Gulcin I, Oktay M, Kufrayvioglu OI, Aslan A, (2002). Determination of antioxidant activity of Lichen Cetraria islandica (L.). Acetylcholine, *Journal of Ethnopharmacology*, 79, 325–329.

Haque M, Haque M E, Rahman M M, 2008. "Antibacterial and cytotoxic activities of *Capparis zeylanica* Linn roots", *Ars Pharmaceutica*, 49, pp.31-37

Hasan MS, Ahmed M I, Mondal S, Uddin S J, Masud M M, Sadhu SK, Ishibashi M, 2006. Antioxidant, antino-ciceptive activity and general toxicity study of *Den-drophthoe falcata* and isolation of quercitrin as themajor component, *Oriental Pharmacy and Experimental Medicine,* 6: 355-60

Khalaf N A , Shakya A K, Othman A L, El-Agbar Z, Farah H, (2008). Antioxidant Activity of Some Common Plants, *Turk J Biol,* 32: 51-55

Kourounakis A P, Galanakis D, Tsiakitzis K, (1999). Synthesis and pharmacological evaluation of novel derivatives of anti-inflammatory drugs with increased antioxidant and anti-inflammatory activities. *Drug Dev. Res.* 47: 9-16.

Ketusing O, (1988). Sunthornphu's Poet About Medicinal Plants. The Memorial Book for 72 Years of Age. Prachachon, Bangkok, p. 8.

Koleva I I, Van Beek T A, Linssen J P H, Groot A De, Evstatieva L N, (2002). Screening of plant extracts for antioxidant activity: a comparative study on three testing methods" *Phytochemical Analysis,* 13: 8-17.

Lee S E, Hwang H J, Ha J.S, (2003). Screening of medicinal plant extracts for antioxidant activity, *Life Science,* 73: 167-179.

Madsen H L, Nielsen B R, Bertelsen G, Skibsted L H, (1996). Screening of antioxidative activity of spices. A comparison between assays based on ESR spin trapping and electrochemical measurement of oxygen consumption. *Food Chemistry,* 57: 331– 337.

Moller J K S, Madsen H L, Altonen T, Skibsted L H, (1999). Dittany (Origanum dictamnus) as a source of water-extractable antioxidants. *Food Chemistry*, 64: 215–219.

Okudu T, Yoshida T, Hatano T, (1994). Food phytochemicals for cancer prevention II. In C. T. Ho, T. Osawa, M.T. Huang and R.T. Rosen (Eds.), Chemistry and antioxidative effects of phenolic compounds from licorice, tea and Compositae and Labiateae herbs (pp. 132-143). Washington, DC: American Chemical Society.

Penchom P, Rungravi T, Jeevan K P, Yasuhiro T, Shigetoshi K, Suwan S T, Hiroshi W (1998), 4 Hydroxybenzoic acid: a hypoglycemic constituent of aqueous extract of *Pandanus odorus* root, *Jurnal of Ethanopharmacology* , Voume 62 (1), 79-84

Phongboonrod S, (1976). Mai thed mueng Thai, Folkloric Uses of Thai and Foreign Medicinal Plants. Chaiwat Press, Bangkok.

Sadhu S K, Okuyama E, Fujimoto H, Ishibashi M, (2003)."Separation of *Leucas aspera,* a medicinal plant of Bangladesh, guided by prostaglandin inhibitory and antioxidant activities" *Chemical & Pharmaceutical Bulletin,* 51: 595-598

Tepe B , Sokmen M, Akpulat H A, Sokmen A, (2006). Screening of the antioxidant potentials of six Salvia species from Turkey. *Food Chem*. 95, 200-204.

Yildirim A, Mavi A, Oktay M, Kara A A, Algur OF, Bilaloglu V, (2000). Comparison of antioxidant and antimicrobial activities of tilia (Tilia argenta Desf Ex DC), sage *(Salvia triloba L.)* and black tea *(Camellia sinensis)* extracts. *Journal of Agricultural and Food Chemistry*, 48: 5030–5034.

Zheng W, Wang S Y, (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food Chemistry*, 49: 5165–5170.

****