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Evaluation of Free Radical Scavenging Activity of *Pandanus odoratissimus*

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Abstract: *Pandanus odoratissimus* is used in traditional medicinal and it is also famous for its fragency. The present study was performed to evaluate the methanolic effect of *Pandanus odoratissimus* (MEPO) against free radical damage. The antioxidant activity of has MEPO been studied using its ability to scavenging DPPH, Nitric acid, superoxide radicals and hydroxyl radicals. The MEPO shows antioxidant activity by 87.52% reducing the DPPH and 73.55% inhibition of nitric acid. The result also indicates maximum inhibition of superoxide radical's inhibition 74.12 and 78.14% inhibition of hydroxyl radicals. The BHT was used as standard.

Key words: Antioxidant, DPPH, superoxide radicals, reducing power assay

INTRODUCTION

Oxidative stress results from an imbalance between the generations of oxygen derived radicals and the organism's antioxidant potential playing an important role in many chronic diseases (Abdollahi *et al*., 2004). There is increasing evidence to show the involvements of free radicals and reactive oxygen species in a variety of disease, they can cause damage to cellular biomolecules such as nucleic acid, protein, lipids and carbohydrates and consequently may adversely affect immune function (Sheetal *et al*., 2008). The efficacy of a plant extract as an antioxidant is best evaluated based on results obtained by commonly accepted assays taking into account different oxidative conditions, system compositions and antioxidant mechanism (Prior and Wu, 2005). It is believed that medicinal plants are a potential source of antioxidant and ROS scavenger molecules (Arora *et al*., 2005). Natural antioxidants tend to be safer and they also possess antiviral, antitumor, hepatoprotective properties (Lim and Mmurtijaya, 2007).

Antioxidants play an important role in the allevation of diabetes due to oxidative stress (Dhanabal *et al*., 2005). Novel natural antioxidant with desired physiochemical properties is in high demand for their application as nutraceuticals and additives (Yu and Zhou, 2004).

Pandan is said to be a restorative, deodorant, indolent and phylactic, promoting a feeling of well-being and acting as a counter to tropical latitude. It may be chewed as a breath sweetener or used as a preservative on foods. It is also said to have healthful properties, including antiviral, anti-allergy, antiplatelet, anti-inflammatory, antioxidant and antitumor. Ayurvedic science finds the medicinal action of the essential oil yielded by the screw pine's highly scented flowers to be useful in headaches, earaches and as a liniment for rheumatic pains. The distilled water made from, the flowers are used for inducing perspiration. It is also prescribed as a stimulant and an antispasmodic. The flowers themselves are powdered and included in medicines, which are either sniffed like snuff or smoked for asthma and other bronchial infections (Keerthikar and Basu, 2000). Hence, the present investigation to study antioxidant property of *Pandanus odoratissimus* is undertaken.

MATERIALS AND METHODS

Collection of plant material: Leaves of the plants *Pandanus odoratissimus* (PO) were collected during March 2008 from Gurmitkal, Gulbarga District North Region of Karnataka, India. The samples were authenticated in Botany Department, Gulbarga University, Gulbarga. Plant materials were dried and stored in shade and were powered to mesh as and required. PO was prepared by equal amount of powders by weight; the study was conduct on May 2008 to December 2008.

Ethylene Diamine Tetra Acetate (EDTA) and Folin Ciocalteu's reagent were purchased from SD fine chemicals, Mumbai, India. 1, 1-Diphenyl-2-picryl hydrazyl (DPPH), riboflavin, Nitro Blue Tetrazolium (NBT) chloride and pyrogallol were purchased from Himedia Ltd, India. Potassium ferricyanide was purchased from Qualigens Fine Chemicals, India. Trichloroacetic acid (TCA) and Iron

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(II) chloride ($FeCl_3$) from E. Merck India Ltd, Indigo Caramine was purchased from SD Fine Chemicals, India.

Preparation of extract: Twenty five gram of the powders of *Pandanus odoratissimus* leaf and its components were extracted separately with various extracts like petroleum ether, Chloroform and Methanol (250 mL) at respective boiling point of extracts. The extract was filtered using Whatman 1 filter paper, pooled and concentrated to dryness under reduced pressure.

Preliminary phytochemical testing: Five hundred milligrams of the dried methanol extract was reconstituted in 10 mL of methanol and it was subjected to preliminary phytochemical testing for the presence of different chemical groups of compounds (Ravishankara *et al*., 2002).

Free radical scavenging activity: A stock solution of DPPH (1.3 mg mL^{-1} in methanol) was prepared such that 75 µL of it in 3 mL methanol gave an initial absorbance of 0.9. Decrease in the absorbance in the presence of sample extract at different concentration was noted after 10 min. EC_{50} was calculated for percentage of inhibition. A 0.1 mM solution of DPPH in methanol was prepared and 1.0 mL of this solution was added to 3.0 mL of control standard Butylated Hydroxyl Toluene (BHT) at difference concentration (25-100 µg mL^{-1}) and test solution at difference concentration (5-100 µg mL^{-1}) in different test tubes. The absorbance was measured at 517 nm.

Nitric oxide scavenging activity: Nitric oxide scavenging activity was measured by the spectrophotometer method (Madan *et al*., 2005). Sodium nitroprusside (5 mmol) in phosphate-buffered saline was mixed with a control without the test compound, but with an equivalent amount of methanol. Test solutions at different concentration (5-100 µg mL^{-1}) were dissolved in methanol and incubated at 25°C for 30 min. After incubation, 1.5 mL of incubated solution was removed and diluted with 1.5 mL of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of the nitrite with sulphanilamide and the subsequent coupling with naphthylethylene diamine dihydrochloride was measured at 546 nm.

Superoxide radical scavenging activity: Assay for superoxide radical scavenging the activity was based on the capacity of the sample to inhibit blue formazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system (Ravishankara *et al*., 2002). The reaction mixture contained 50 nm phosphate buffer (pH 7.6), 20 µg riboflavin, 12 mM EDTA, NBT 0.1 mg/3 mL, added in that sequence. The reaction was started by illuminating the reaction mixture with different concentrations of sample extract for 150 sec. Immediately after illumination, the absorvance was measured at 590 nm and EC_{50} was calculated. Methanol was used for blank reading.

Hydroxyl radical scavenging activity: The scavenging capacity for hydroxyl radical was measured according to the modified method (Rajeshwar *et al*., 2005). The assay was performed by adding 0.1 mL EDTA, 0.01 mL of $FeCl_3$, 0.01 mL of H_2O_2, 0.36 mL of deoxyriboase, 1.0 mL of test solution (5-100 µg mL^{-1}) dissolved in distilled water, 0.33 mL of phosphate buffer (50 mM, pH 7.4) and 0.1 mL of ascorbic acid in sequence. The mixture was then incubated at 37°C for 1 h a 1.0 mL portion of the incubated mixture was mixed with 1.0 mL of 10% TCA and 1.0 mL of 0.5% TBA to develop the pink chromogen. The optical density was measured at 532 nm.

Statistical analysis: The results are presented as Mean±SEM. All the parameters were analyzed by using student's t-test. The data was considered as significant, if the p-value is $p<0.05$.

RESULTS

In the present study, preliminary phytochemical studies for the, test of, steroids, terpenoids and glycosides were found positive in the extracts. Alkaloids, tannin flavoniodes and phenolics were showed high presence in all the extract. The total phenolic content in the aqueous extract was ranged from 3.5 to 10.8% w/w. phenolics are the largest groups of phytochemicals and have been said to account for most of the antioxidant activity of plant extracts.

Inhibition of DPPH radicals: The potential decrease in the concentration of DPPH radical is due to the scavenging ability of MEPO and BHT (standard reference) showed the significant free radical scavenging activity 91.55 and 87.52% of inhibition, respectively, at 100 µg mL^{-1}. The (IC_{50}) inhibitory concentration at which there is 55% reduction of free radical of MEPO was found to be 36 µg mL^{-1} (Fig. 1).

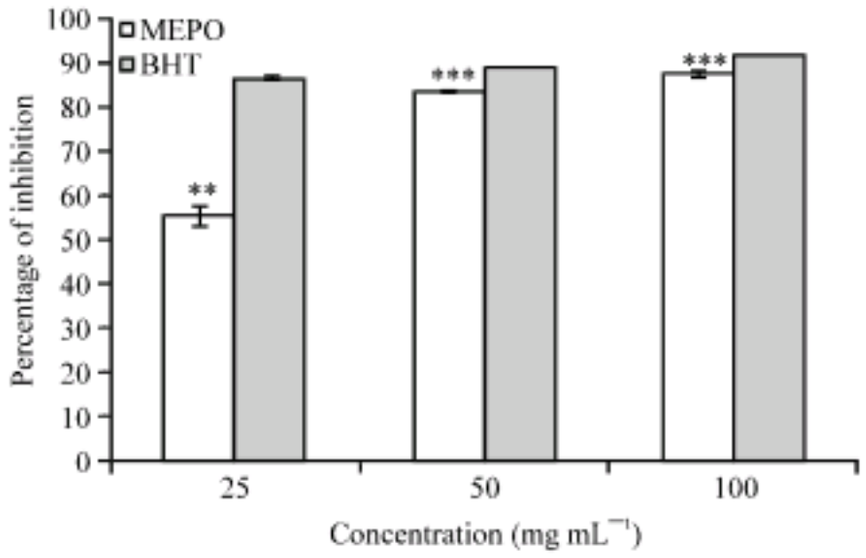

Fig. 1: Effect of MEPO compared with standard BHT for Inhibition of DPPH. Values are Mean±SEM, 6 independent analysis. **p<0.01, ***p<0.001

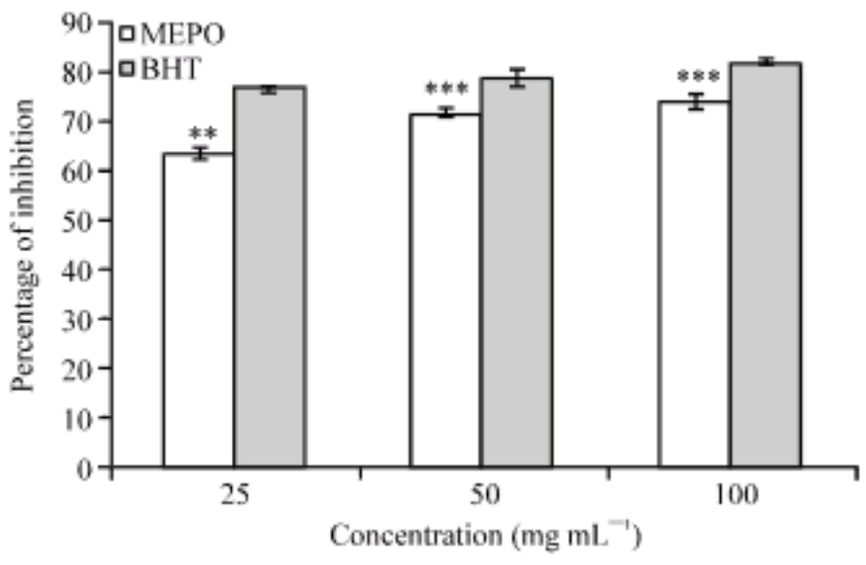

Fig. 2: Effect of MEPO compared with standard BHT for Nitric oxide activity. Values are Mean±SEM, 6 independent analysis. **p<0.01, ***p<0.001

Nitric oxide scavenging activity: The scavenging activity of nitric oxide by MEPO and BHT was mainly concentration depend on various concentrations of the extract. There was a moderate inhibition of nitric oxide formation, with the maximum inhibition 73.50 and 81.35% at 100 μg mL^{-1} of *Pandanus odoratissimus* and BHT was observed (Fig. 2).

Superoxide radicals scavenging: A moderate inhibition of the superoxide radicals was observed with 100 μg mL^{-1} each of MEPO and BHT 74.12 and 79.67%, respectively (Fig. 3).

Hydroxyl radical activity: The MEPO and BHT have shown a significant inhibition on hydroxyl radical and iron (II)-dependent deoxyribose damage at all concentrations. The percentage of inhibition of hydroxyl radical being 78.17% and BHT 74.30%, respectively at 100 μg mL^{-1} (Fig. 4).

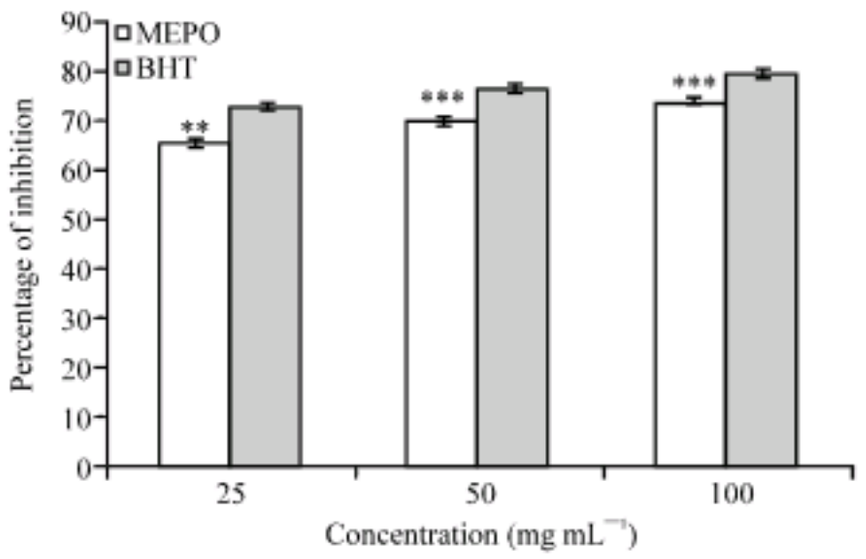

Fig. 3: Effect of MEPO compared with standard BHT for Superoxide radicals scavenging activity. Values are Mean±SEM, 6 independent analysis. **p<0.01, ***p<0.001

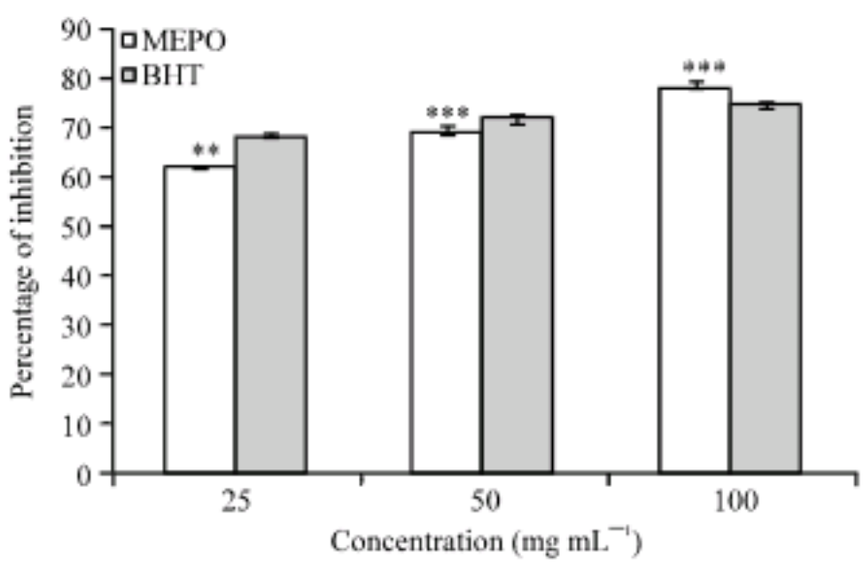

Fig. 4: Effect of MEPO compared with standard BHT for Hydroxyl radical activity. Values are Mean±SEM, 6 independent analysis. **p<0.01, ***p<0.001

DISCUSSION

Oxidative stress, in a large quantities of Reactive Oxygen Species (ROS) like hydrogen peroxide, superoxide (O^{-}_{2}), hydrogen radical (OH^{-}), singlet oxygen generated is one of the earliest responses to stress. These ROS have a role in disease and aging in animals (Halliwell and Gutteridge, 1998). The antioxidative system protects the organism against ROS induced oxidative damage. There are restrictions on the use of synthetic antioxidants, such as BHT, because they are suspected to be carcinogenic (Govindarajan *et al*., 2003). Therefore, natural antioxidants have gained importance.

The DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm. Which is induced by antioxidants? DPPH radicals react with suitable reducing agents and then electrons become paired off and the solution loses

color stoichometrically with the number of electrons taken up (Blois, 1958). The significant decrease in the concentration of the DPPH radical is due to the scavenging ability of *Pandanus odoratissimus*.

The nitric oxide was generated by sodium nitroprusside and measured by the Greiss reduction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produces nitrates ions that can be estimated by using Greiss reagent. Scavengers of nitric oxide compete with the oxygen reduced production of nitric oxide.

There was a moderate inhibition of the superoxide radical, with the maximum inhibition being 66% at 1 mg mL^{-1} extract concentration. Superoxide anion is oxygen centered radical with selective reactivity. This species is produced by a number of enzyme systems in auto-oxidation reaction and by nonenzymatic electron transfers that univalently reduce molecular oxygen. It can also reduce certain iron complex such as cytochrome (Gülçin *et al*., 2003).

The potentially reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins. The effect of *Pandanus odoratissimus* on the inhibition of free radical mediated deoxyribose damage was assessed by means of iron (II) dependent DNA damage assay, which showed significant results (Jornot *et al*., 1998).

CONCLUSION

Pandanus odoratissimus leaf extract demonstrated moderate activity of antioxidant, reducing power and scavenging activity. Purification of the extract may lead to increase activity in its bioactive compounds. The antioxidants activity of MEPO may be due to its proton donating capability as shown in the DPPH radical scavenging results. Acting as an electron donor that can react with free radicals its converts them to more stable products and terminate radical chain reactions.

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