International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Suppl 3, 2011

Research Article

COMPARATIVE *IN VITRO* ANTIOXIDANT ACTIVITY OF DIFFERENT PARTS OF *COCOS NUCIFERA* (LINN.) ON REACTIVE OXYGEN AND NITROGEN SPECIES

SAGAR NASKAR[1, 2*], UPAL K. MAZUMDER[1], GOUTAM PRAMANIK[2], ASIS BALA[1], PALLAB K. HALDAR, AMINUL ISLAM[1] AND MALAYA GUPTA[1]

[1]Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India, [2]Bengal College of Pharmaceutical Sciences and Research, Durgapur, India. Email: sagar_n2007@yahoo.co.in

Received: 18 Feb 2011, Revised and Accepted: 19 March 2011

ABSTRACT

The present study was aimed to investigate the comparison of antioxidant activities of different parts of the hydromethanol extract of *Cocos nucifera* (Linn.) belongs to Arecaceae family. The portions taken for the study were young spadix and young immature green fruits. The antioxidant activities of extracts have been evaluated by using a range of *in vitro* free radical scavenging assay models and reductive ability was accounted from the conversion of Fe^{3+}/ferricyanide complex to the Fe^{2+} form. The IC_{50} values were found to be 937 and 37.83 µg/ml in DPPH, 490 and 66.6 µg/ml in Superoxide, 141.67 and 37.5 µg/ml in Nitric oxide and 558 and 14.83 µg/ml in Hydroxy radical scavenging model for spadix and young immature green fruits, respectively. The free radical scavenging and antioxidant activities may be attributed to the presence of phenolic compound and flavonoid. Pyrocatechol content were found to be 34.62 and 77.77 µg/mg and gallic acid content were 6.71 and 14.87 µg/mg of the spadix and young green fruits, respectively. Flavonoid compounds were found to be 6.945 and 3.47 µg/mg of the spadix and young green fruits, respectively. The results obtained in the present study indicate that the spadix as well as young immature green fruit of *Cocos nucifera* Linn is a potential source of natural antioxidant and the potency of green fruit was high than the spadix.

Keywords: *Cocos nucifera,* 1, 1-Diphenyl-2-picrylhydrazyl (DPPH), Antioxidant activity, Phenolic compound, Flavonoid.

INTRODUCTION

There is an increased evidence for the participation of free radicals in the etiology of various diseases like cancer, diabetes, cardiovascular diseases, autoimmune disorders, neurodegenerative diseases, aging etc[1]. A free radical is defined as any atom or molecule possessing unpaired electrons. Antioxidants are agents which scavenge the free radicals and prevent the damage caused by reactive oxygen species (ROS), reactive nitrogen species (RNS). ROS is composed of superoxide anion (O^{2-}), hydroxyl (OH·), hydroperoxyl (OOH·), peroxyl (ROO·), alkoxyl (RO·) radicals non free radicals are hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3) singlet oxygen ($^1O_2$). RNS are mainly nitric oxide (NO·), peroxynitrite (ONOO·) nitrogen dioxide (NO_2). Antioxidants can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells prevent damage to lipids, proteins, enzymes, carbohydrates DNA. A wide range of antioxidants from both natural and synthetic origin has been proposed for use in the treatment of various human diseases. There are some synthetic antioxidant compounds such as butylated hydroxytoluene, butylated hydroxyanisole tertiary butylhydroquinone which are commonly used in processed foods. However, it has been suggested that these compounds have shown toxic effects like liver damage and mutagenesis. Flavonoids and some other phenolic compounds of plant origin have been reported as scavengers of free radicals. Hence, nowadays search for natural antioxidant source is gaining much importance.

The Coconut (*Cocos nucifera* Linn. belongs to Arecaceae family)[2] is native to coastal areas of Southeast Asia. The flowers are unisexual, stalkless and sprout from a fleshy branched panicle or spadix in the axle of a huge, woody, fibrous bract (spathe). The fruit is a three-sided drupe. Its wall, or pericarp, consists of three distinct layers: a smooth thin rind or exocarp, which is green to begin with, but changes to brown or reddish-brown on maturity; a large brown fibrous middle region (or the mesocarp) forming the husk; and, a hard stony endocarp which forms the coconut shell, enclosing the seed inside. The roots are astringent, diuretic and anthelmintic, and are useful in pharyngodynia, uterine disorders, blennorrhagia, bronchitis, hepatopathy, strangury and helmenthiasis. The juice of the young spadix when fresh is sweet, refrigferant, asperient, aphrodisiac diarrhea, dysentery, diabetes, haemoptysis, strangury, leprosy and general debility. The shell is cooling, diuretic and deodorant, and is good for hyperdipsia, strangury and halitosis. The kernel is sweet, cooling, oleaginous, indigestible, appetizer, aphrodisiac, laxative and tonic and is useful in bronchitis, vitiated condition of pitta, hyperdipsia, tumours, skin diseases, eruptive fever, haemoptysis and general debility. The water is sweet, cooling digestive[3].

The objective of the present study was to investigate the free radical scavenging and reductive ability of different parts of hydromethanol extract of *Cocos nucifera* Linn. by using a range of *In vitro* antioxidant and reductive ability models as well as determination of total phenolic, flavonoid content in order to evaluate a relationship between the antioxidant activity and the phytochemical constituents.

MATERIALS AND METHODS

Plant material

The spadix and young green fruit of coconut (*Cocos nucifera* Linn.) were collected from South 24 Parganas, West Bengal. The materials were extracted with 20% methanol by cold maceration process and then filtrate extracts were dried on rotary vacuum evaporator and water bath respectively. Dried extracts were kept in refrigerator and used for further study.

Evaluation of antioxidant activity

DPPH radical scavenging activity

DPPH radical scavenging activity was measured using the method of Cotelle et al.[4] with some modifications. 2 ml of reaction mixture containing 1 ml of DPPH (100 µM in methanol) 1 ml of test solution, at various concentrations of the extract fractions was incubated at 37°C for 30 min absorbance of the resulting solution was measured at 517 nm using Beckman model DU-40 spectrophotometer. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with extract) using the following equation:

Percentage inhibition = (1– absorbance of test/absorbance of control) × 100 …… (1)

Nitric oxide scavenging activity

In aqueous solution at physiological pH, sodium nitroprusside generates nitric oxide, which interacts with oxygen to produce nitrite ions[5], which can be measured by the Griess reaction. 1ml of

10 mM sodium nitroprusside was mixed with 1 ml of test solution of different concentrations in phosphate buffer (pH 7.4) and the mixture was incubated at 25°C for 150 min. From the incubated mixture, 1 ml was taken out 1 ml of Griess' reagent (1% sulphanilamide, 2% o-phosphoric acid 0.1% naphthyl ethylene diamine dihydrochloride) was added to it. Absorbance of the chromophore formed by the diazotization of nitrite with sulfanilamide subsequent coupling with naphthyl ethylene diamine dihydrochloride was read at 546 nm. The percentage inhibition was calculated by comparing the results of the test with those of the control using Eq. (1).

Hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell et al[6]. Stock solutions of EDTA (1 mM), $FeCl_3$ (10 mM), ascorbic acid (1 mM), H_2O_2 (10 mM), deoxyribose (10 mM), were prepared in distilled deionized water.

The assay was performed by adding 0.1 ml EDTA, 0.01 ml of $FeCl_3$, 0.1 ml H_2O_2, 0.36 ml of deoxyribose, 1 ml of the extract of different concentration 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 hr. 1 ml portion of the incubated mixture was mixed with 1 ml of 10% TCA 1 ml of 0.5% TBA (in 0.025 M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging activity of the extract is reported as % inhibition of deoxyribose degradation is calculated by using Eq. (1).

Superoxide radical scavenging activity

Superoxide anion scavenging activity was measured according to the method of Robak and Gryglewski[7] with some modifications. All the solutions were prepared in 100 mM phosphate buffer (pH 7.4). 1ml of nitroblue tetrazolium (NBT, 156 µM), 1 ml of reduced nicotinamide adenine dinucleotide (NADH, 468 µM) 1 ml of test solution of different concentrations were mixed. The reaction was initiated by adding 100 µl of phenazine methosulphate (PMS, 60 µM). The reaction mixture was incubated at 25°C for 5 min, followed by measurement of absorbance at 560 nm. The percentage inhibition was calculated by using Eq. (1).

Reductive ability

Reducing power of the test samples was determined on the basis of the ability of their antioxidant principles to form colored complex with potassium ferricyanide, TCA and $FeCl_3$ it was measured by the method reported by Jayprakash et al[8]. 1 ml of different concentrations (25 to 900 µg/ml) of the extract fractions were mixed with potassium ferricyanide (2.5 ml, 1%) 2.5 ml of phosphate buffer (pH 6.6). The mixture was incubated at 50°C for 20 min. 2.5 ml TCA (10%) was added to it and centrifuged at 3000 rpm for 10 min. 2.5 ml of supernatant was taken and 2.5 ml water and 0.5 ml $FeCl_3$ (0.1%) were added to it. The absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated higher reducing power.

Estimation of total phenolic (pyrocatechol and gallic acid) compounds

Total soluble phenolic in the extracts were determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton[9]. Briefly, 0.1 ml of extract in distilled water (contains 1mg of each extract) was transferred into 100 ml Erlenmeyer flask then final volume was adjusted to 46 ml by addition of distilled water. Afterwards, 1 ml of Folin – Ciocalteu reagent (FCR) was added to this mixture and after shaking for 3 min. 3 ml of Na_2CO_3 (2%) was added. Subsequently, mixture was shaken for 2 hours at room temperature then absorbance was measured at 760 nm. The concentrations of total phenolic compounds in extracts were by using Eqs. (2) and (3) that were obtained from standard graph.

Absorbance = 0.001 × pyrocatechol (µg) + 0.0033 …….. (2)

Absorbance = 0.0053× gallic acid (µg) -0.0059 …….. (3)

Estimation of total flavonoid content

The total flavonoid content was determined using the Dowd method[8]. 2 ml of 2 % aluminium chloride ($AlCl_3$) in methanol was mixed with the same volume of the extract solution (0.1 mg/mL). Absorption readings at 415 nm using spectrophotometer were taken after 10 minutes against a blank sample consisting of a 2 ml extract solution with 2 ml methanol without $AlCl_3$. The total flavonoid content was determined using a standard curve with quercetin (25 - 200 µg/2ml methanol) as the standard. Total flavonoid content is expressed as µg of quercetin equivalents (QE)/mg of extract.

Statistical analysis

All the results were shown as average ± S.E.M. IC_{50} value was determined to be the effective concentration at which free radicals were scavenged by 50%. The IC_{50} value was obtained by interpolation from linear regression analysis.

RESULTS AND DISCUSSION

DPPH radical scavenging activity

DPPH assay is one of the most widely used methods for screening antioxidant activity of plant extracts. The extract demonstrated H-donor activity. DPPH is a stable, nitrogen-centered free radical which produces violet colour in ethanol solution. It was reduced to a yellow coloured product, diphenylpicryl hydrazine, with the addition of the extract in a concentration-dependent manner. IC_{50} values were found to be 937 and 37.83 µg/ml in DPPH model for spadix and young immature green fruits, respectively.

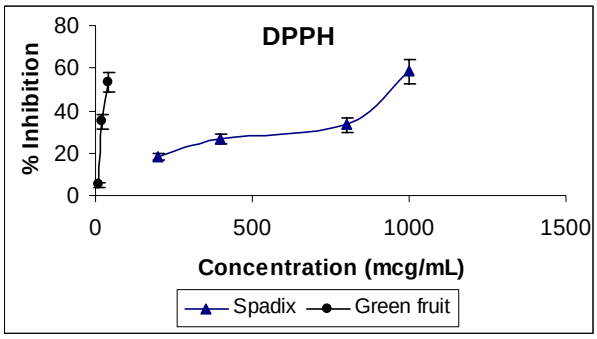

Fig. 1: DPPH free radical scavenging activity of spadix and green fruit extract of *Cocos nucifera*

Nitric oxide scavenging activity

Scavenging of nitric oxide radical was based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent. The extract decreases the amount of nitrite generated from the decomposition of sodium nitroprusside in vitro. The IC_{50} values were found to be 141.67 and 37.5 µg/ml in Nitric oxide scavenging model for spadix and young immature green fruits, respectively.

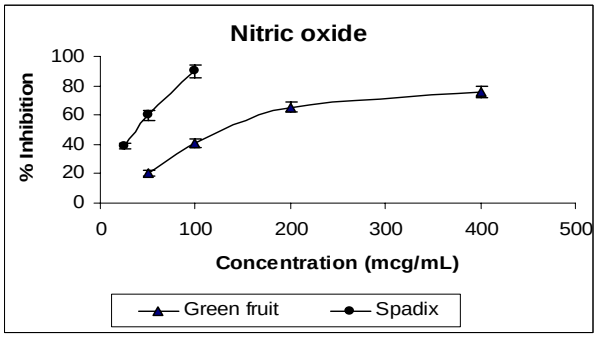

Fig. 2: Nitric oxide free radical scavenging activity of spadix and green fruit extract of *Cocos nucifera*

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was quantified by measuring the inhibition of the degradation of deoxyribose by the free radicals generated by the Fenton reaction. The oxygen derived hydroxyl radicals along with the added transition metal ion (Fe^{2+}) causes the degradation of deoxyribose into malondialdehyde which produces a pink chromogen with thiobarbituric acid. The IC_{50} values were found to be 558 and 14.83 µg/ml in Hydroxy radical scavenging model for spadix and young immature green fruits, respectively.

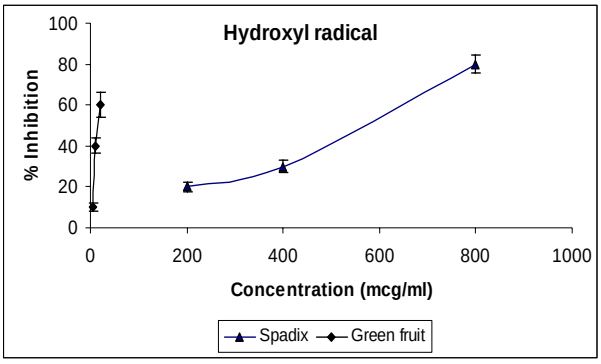

Fig. 3: Hydroxyl free radical scavenging activity of spadix and green fruit extract of *Cocos nucifera*

Superoxide radical scavenging activity

Superoxides are produced from molecular oxygen due to oxidative enzyme of body as well as via non-enzymatic reaction such as autoxidation by catecholamines. In the present study, superoxide radical reduces NBT to a blue colored formazan that is measured at 560 nm. The IC_{50} values were found to be 490 and 66.6 µg/ml in Superoxide radical scavenging model for spadix and young immature green fruits, respectively.

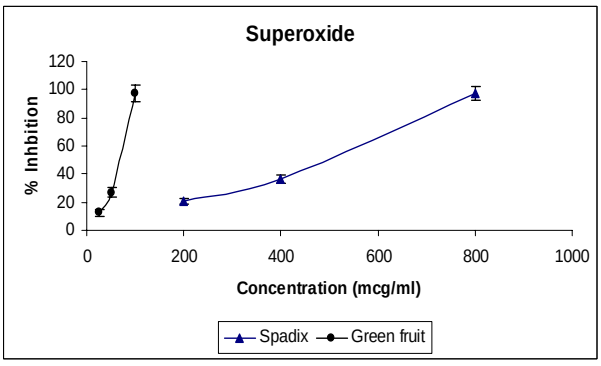

Fig. 4: Super oxide free radical scavenging activity of spadix and green fruit extract of *Cocos nucifera*

Reductive ability

Like the antioxidant activity, the reducing power increased with increasing amount of the extract. For the measurement of the reductive ability, the Fe^{3+}-Fe^{2+} transformation was investigated in presence of the extract. Presence of reductants causes the reduction of the Fe^{3+}/ferricyanide complex to the Fe^{2+} form. This Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Higher absorbance of the reaction mixture indicated higher reducing power.

Estimation of total phenolic (pyrocatechol and gallic acid) compounds

The free radical scavenging and antioxidant activities may be attributed to the presence of phenolic compound and flavonoid. Pyrocatechol content were found to be 34.62 and 77.77 µg/mg and gallic acid content were 6.71 and 14.87 µg/mg of the spadix and young green fruit extracts, respectively. Total phenolic assay by using Folin-Ciocalteu reagent is a simple, convenient reproducible method. It is employed routinely in studying phenolic antioxidants.

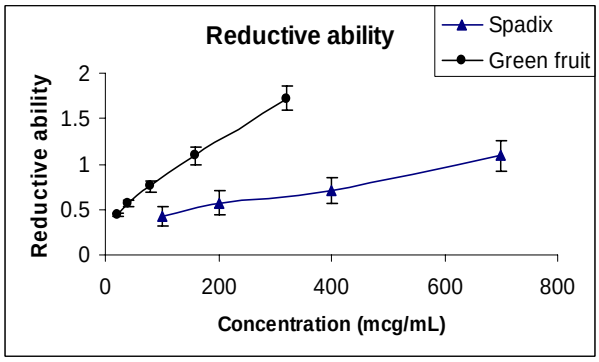

Fig. 5: Reductive ability of spadix and green fruit extract of *Cocos nucifera*

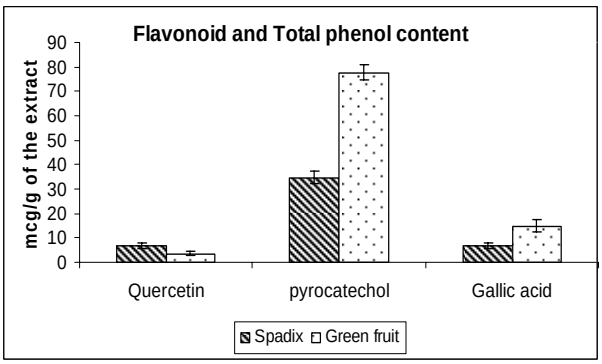

Fig. 6: Total phenolic and flavonoid content of spadix and green fruit extract of *Cocos nucifera*

Estimation of total flavonoid content

Flavonoids are large class of benzo-pyrone derivatives, ubiquitous in plants exhibit antioxidant activity. Flavonoid compounds were found to be 6.945 and 3.47 µg of quercetin equivalents (QE) per mg of the spadix and young green fruit extracts, respectively. The antiradical property of flavonoids is directed mostly toward hydroxyl, superoxide as well as peroxyl and alkoxyl radicals. Furthermore, as these compounds present a strong affinity for iron ions (which are known to catalyze many processes leading to the appearance of free radicals), their antiperoxidative activity could also be ascribed to a concomitant capability of chelating iron.

CONCLUSION

Free radicals are known to play a definite role in a wide variety of pathological manifestations of pain, inflammation, cancer, diabetes, alzheimer, hepatic damage etc. antioxidants fight free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms. Hence, the present investigation suggests that extracts show good antioxidant activity, reducing power, free radical scavenging activity. Phytochemical screening of the crude extract reveals the presence of flavonoids, saponins, tannins. Thus these in vitro antioxidant potential of extracts may be due to the presence of these phytoconstituents.

ACKNOWLEDGEMENT

The authors are grateful to Jadavpur University for providing research facilities.

REFERENCES

1. Beckman KB, Ames BN. The free radical theory of aging matures. Physiological Reviews. 1998; 78: 547-81
2. Malu PC, Deasai AG, Joshi GD. Evaluation of Coconut (Cocos nucifera L.) Cultivars for Tender Nut water. Indian Coconut Journal. 2004; XXXIV: 5-12
3. Vaidyaratnam PS. Indian medicinal plants. Vol.2. Chennai: Orient Longman Private Limited; 1994
4. Cotelle N, Bemier JL, Catteau JP, Pommery J, Wallet JC, Gaydou EM. Antioxidant properties of hydroxyl-flavones. Free Radic. Biol. Med. 1996; 20: 35-43.
5. Chou HJ, Kuo JT, Lin ES. Comparative antioxidant properties of water extracts from different parts of Beefsteak plant (Perilla frutescens). J. Food Drug Ana. 2009; 17: 489-496.
6. Halliwell B. Antioxidants in human health and disease. Annual Review of Nutrition. 1996; 16:33-50.
7. Robak J, Gryglewski RJ. Flavonoids are scavengers of superoxide anions. Biochem. Pharmacol. 1988; 37: 837-841.
8. Naskar S, Islam A, Mazumder U, Saha P, Haldar PK, Gupta M. *In Vitro* and *In Vivo* Antioxidant Potential of Hydromethanolic Extract of *Phoenix dactylifera* Fruits. J. Sci. Res. 2010; 2: 144-157.
9. Singleton VL, Orthofer R, and Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. Methods Enzymol. 1999; 299: 152-178.