Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 2, 2012

Research Article

IN VITRO FREE RADICAL SCAVENGING ACTIVITY OF *ANANUS COMOSUS* (L.) MERRILL PEEL

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Received: 13 Dec 2011, Revised and Accepted: 19 Feb 2012

ABSTRACT

Ananas comosus (L.) Merrill is belonging to the family Bromeliaceae and it is an important tropical and subtropical plant widely cultivated in the tropical areas of the world. The ethanolic extract of the *Ananus comosus* peel was screened for *in vitro* antioxidant activity. The free radical activity of the ethanolic extract was assayed using 1,1-diphenyl-2-picryl hydroxyl (DPPH), 2,2' azinobis-3 ethylbenzothiozoline-6 sulfonic acid ($ABTS^+$) cation decolourization test, hydroxyl radical (OH^-), hydrogen peroxide assay (H_2O_2), nitric oxide radical inhibition activity (NO), superoxide radical, ferric reducing antioxidant power assay (FRAP), reducing power activity, total antioxidant capacity assay and lipid peroxidation inhibition activity using established assay procedure. The ethanolic extract exhibited high antiradical activity against the above mentioned radicals. The percentage of inhibition was increased in a dose dependent manner. The antioxidant activity of the extract was compared with that of standard butylated hydroxyl toluene (BHT) and vitamin C. In conclusion, the results presented in the peel of *Ananus comosus* have a strong antioxidant property against free radicals and it may serve as a good pharmacological property.

Keywords: *Ananas comosus*, Bromeliaceae, *In Vitro* antioxidant activity, Antiradical, Free radicals

INTRODUCTION

Free radical is any atom with atleast one unpaired electron in the outermost shell and is capable of independent existence. Free radicals and other reactive species produced during aerobic metabolism in the body can cause oxidative damage of amino acids, lipids, proteins and DNA[1]. It has been established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others. Interestingly the body possesses defense mechanisms against free radical induced oxidative stress which involve preventative mechanisms, repair mechanisms, physical effects and antioxidant defenses. Mainly the antioxidant substances block the action of free radicals which have been implicated in the pathogenesis of many diseases including atherosclerosis, ischemic heart disease, cancer, Alzheimer's disease, Parkinson's disease and in the aging process[2].

Antioxidants may protect the body against ROS toxicity either by preventing the formation of ROS, by bringing interruption in ROS attack, by scavenging the reactive metabolites or by converting them to less reactive molecules. The antioxidant capacity gives information about the duration while the activity describes the starting dynamics of antioxidant action. Therefore the uses of antioxidants, both natural and synthetic are gaining wide importance in prevention of diseases[3].

Ananas comosus (L.) Merrill is belonging to the family Bromeliaceae and it is an important tropical and subtropical plant widely cultivated in the tropical areas of the world. Its fruit is consumed fresh or canned as a commercial product in many countries[4]. Pineapple has also been known for a number of beneficial biological activities such as antioxidative, anti-browning, anti-inflammatory and anti-platelet activities. The enzyme complex of *A. comosus* called bromelain is known for its clinical applications particularly modulation of tumor growth, blood coagulation and anti-inflammatory effect[5]. Pineapple has been extensively used in foods or for health benefits. The present study was aimed to evaluate the in vitro free radical scavenging activity of *Ananus comosus* (L.) Merrill peel.

MATERIALS AND METHODS

Collection of Plant material

Fresh pineapple peel was collected from Coimbatore, Tamil Nadu, India. The plant was authenticated by Dr. G.V.S Moorthy, Botanical survey of India, TNAU Campus, Coimbatore and the voucher specimen No.BSI/SRC/5/23/2011/Tech-515. Fresh peel sample was washed under running tap water, air dried, and then homogenized to fine powder and stored in airtight bottles.

Sample Extraction

100g of dried plant powder was extracted in 500ml of ethanol in a water shaker for 72hrs. Repeatedly extraction was done with the same solvent till clear colorless solvent is obtained. Obtained extract was evaporated to dryness by using a rotary vacuum evaporator at 40-50^{o}C and stored at 0-4^{o}C in an air tight container.

In Vitro antioxidant assays

DPPH radical scavenging assay was estimated by Blois method[6], $ABTS^+$ radical activity was estimated by Pellegrini et al. [7]. Hydroxyl Radical Scavenging assay was estimated by Smirnoff method[8]. Hydrogen Peroxide Radical Scavenging assay was determined by replacement titration method of[9]. Super Oxide radical scavenging assay was determined by Liu[10]. Nitric Oxide Scavenging assay was determined according to the method of[11]. Reducing power was determined using[12]. Ferric reducing antioxidant power (FRAP) was estimated by Benzie and Strain method[13], total antioxidant capacity assay was determined by[14] and Anti-lipid peroxidation (Thio barbituric acid method) was determined according to the method given of[15].

RESULTS AND DISCUSSION

Antioxidant properties, especially radical scavenging activity is very important, due to the deleterious role of free radicals in plants and in biological systems. Diverse methods are currently used to assess the antioxidant activity of *Ananus comosus* peel in ethanolic extract. DPPH is used for the evaluation of antioxidant capacity in a short time and frequently applied for testing food products. The reduction capability of the DPPH radical is determined by its decreased absorbance at 517nm as induced by natural antioxidants[16]. The DPPH radical scavenging (%) activity of *Ananus comosus* extract, compared to BHT was shown in Figure 1. The concentrations providing 50% inhibition (IC_{50}) value of the extract was found to be 1.32 mg/ml. The highest scavenging activity of ethanolic extract was found to be 87.10% at a concentration 2.5 mg/ml.

$ABTS^+$ radical, a protonated radical has characteristic absorbance maxima at 734 nm which decreases at the scavenging of proton radical which is known as excellent substrate for peroxidases frequently used to study antioxidant properties of natural compounds[17]. In order to evaluate the antioxidant potency through free radical scavenging by *Ananus comosus* extract, the change of optical density of $ABTS^+$ radicals was monitored and vitamin C was used as standard antioxidant. Figure 2 depicts the $ABTS^+$ radical

scavenging activity of ethanolic extract as well as standard compound. The percentage inhibition of the extract was found to be 62.55% when compared with standard as 59.57% and IC_{50} value was found to be 1.21 mg/ml and 1.63 mg/ml respectively. Thus the radical-scavenging capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

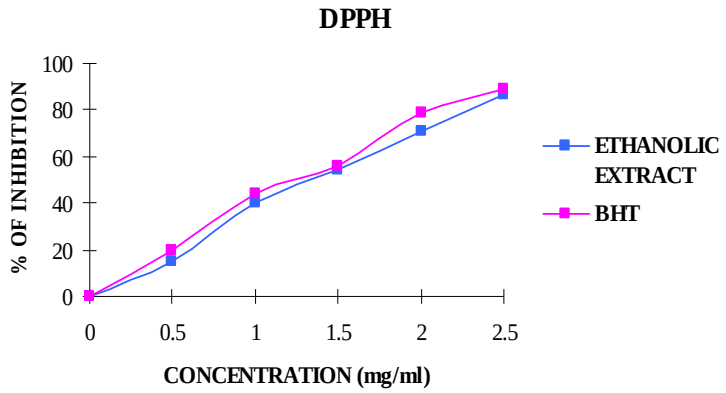

Fig. 1: DPPH radical scavenging activity.

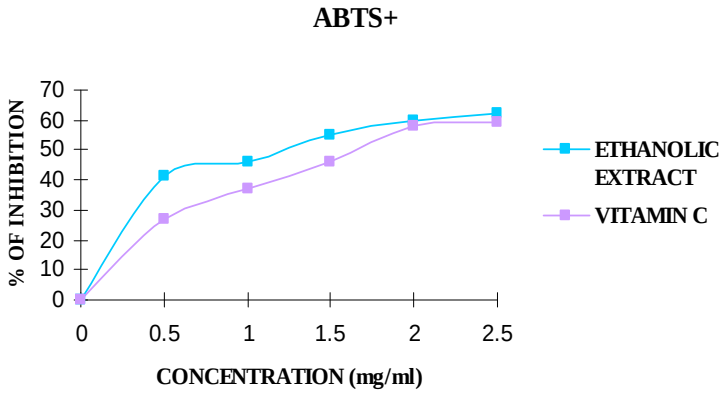

Fig. 2: $ABTS^+$ radical scavenging activity.

Hydroxyl radicals were generated from the substrate deoxyribose by the reaction of ferric- EDTA together with H_2O_2. When the peel extract was incubated with the above reaction mixture, it can prevent the damage against sugar[18]. The results are shown in the figure 3. The scavenging activity of ethanolic extract was found to be 80% and standard antioxidant vitamin C showed the percentage inhibition of 87% at 2.5 mg/ml concentration and the IC_{50} value was found to be 1.32 mg/ml for extract and 1.42 mg/ml for standard. The extract and vitamin C exhibited strong scavenging effects for hydroxyl radicals which could inhibit lipid damage at different concentrations.

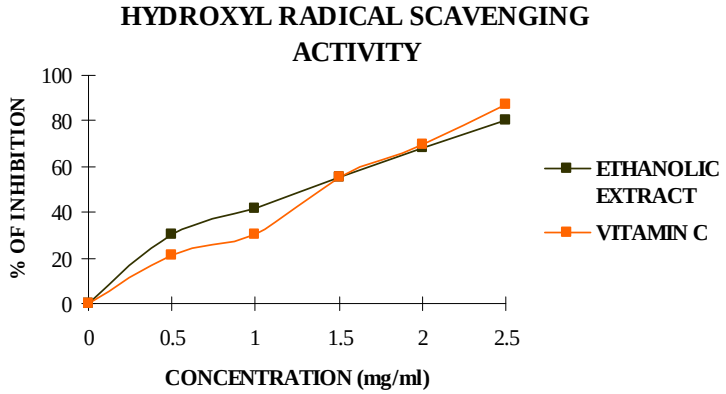

Fig. 3: Hydroxyl radical scavenging activity.

Hydrogen peroxide (H_2O_2) is a byproduct of respiration and is made in all living cells. Hydrogen peroxide is harmful and must be removed as soon as it is produced in the cell. Cells make the enzyme catalase to remove hydrogen peroxide.

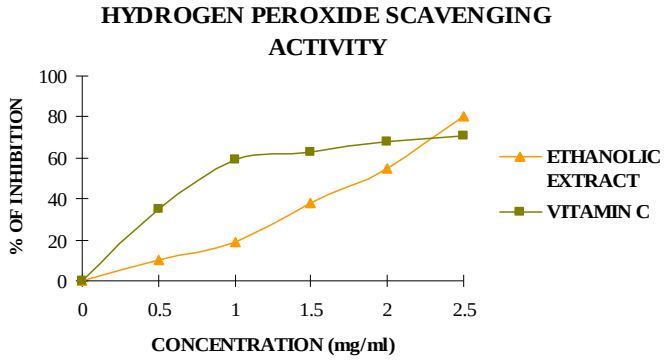

Fig. 4: Hydrogen peroxide radical scavenging activity.

Hydrogen peroxide scavenging depends upon the phenolic content of the extract which can donate electrons to H_2O_2 thus neutralizing it in to water[19]. The scavenging of nitric oxide by fruit extract increased in a dose dependent manner as illustrated in (Figure 4). The results were expressed as IC_{50} values. The percentage inhibition of ethanolic extract was found to be 80.13% at 2.5 mg/ml concentration when compared with standard antioxidant Vitamin C (71.34%). The IC_{50} value was found to be 1.82 mg/ml and 0.42 mg/ml. Thus the ethanolic extract of the *Ananus comosus* peel was capable of scavenging H_2O_2 in a dose dependent manner.

Nitric oxide is a very unstable species, so under aerobic condition it can react with O_2 to produce its stable products such as nitrate and nitrite through intermediates NO_2, N_2O_4. The nitric oxide radical scavenging activity was estimated by using Griess reagent. In the presence of a scavenging test compound, the amount of nitrous acid will decrease and can be measured at 546nm[20]. The nitric oxide radical scavenging activities of *Ananus comosus* peel extract were shown in Figure 5. The IC_{50} value of the extract was found to be 1.31 mg/ml, when compared with standard vitamin C exposed as 1.4 mg/ml.

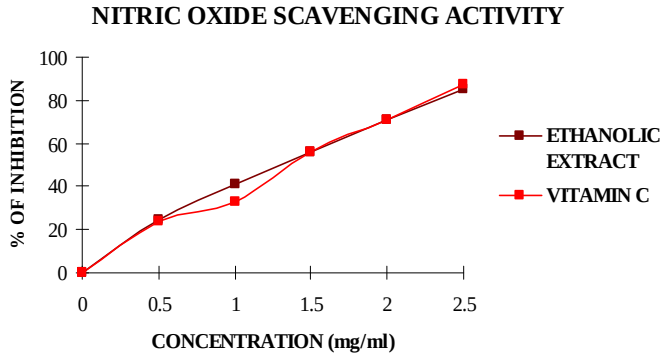

Fig. 5: Nitric oxide radical scavenging activity.

The percentage of inhibition of the extract showed 85.21%. Hence the ethanolic extract of *Ananus comosus* has better nitric oxide radical scavenging activity in competing with oxygen to react with nitric oxide and thus the inhibition of generation of anions.

Superoxide anion is a relatively weak oxidant, but it can generate more dangerous species, including singlet oxygen and hydroxyl radicals, which could cause damage to tissues[21]. In the present study, superoxide radical reduces NBT to a blue colored formazan that is measured at 560 nm. Figure 6 shows the superoxide scavenging effect of *Ananus comosus* peel extract.

The increase of percentage scavenging activity thus indicates the consumption of superoxide anion in the reaction mixture by the plant extracts. Maximum percentage scavenging activity showed by extract is 83.24% at 2.5 mg/ml and the IC_{50} value was found to be 1.3 mg/ml and vitamin C 0.83 mg/ml respectively. Thus the extract has ability to reduce the superoxide radical in a dose dependent manner.

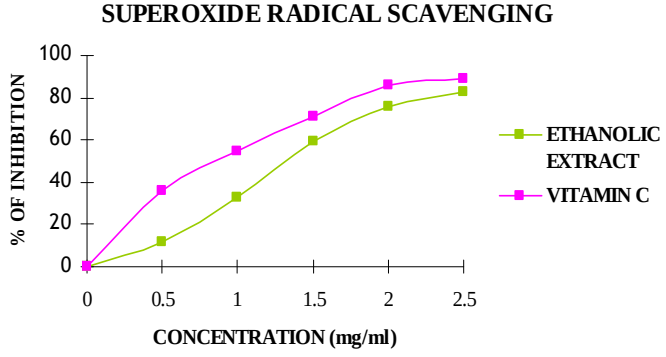

Fig. 6: Superoxide radical scavenging activity.

In FRAP assay the ability of plant extract to reduce ferric ions was determined. FRAP assay measures the changes in absorbance at 593 nm owing to the formation of blue colored Fe – tripyridyltriiazine compound from the colourless oxidized Fe form by the action of electron donating antioxidants[22]. The ethanolic extract showed the hydrogen donating ability have 0.92 mg/ml at 2.5 mg/ml concentration which was compared with the standard antioxidant vitamin-C showed 0.96mg/ml (Figure 7). Since FRAP assay is easily reproducible and linearly related to molar concentration of the antioxidant present, it can be reported that ethanolic extract may act as free radical scavenger, capable of transforming reactive free radical species into stable nonradical products.

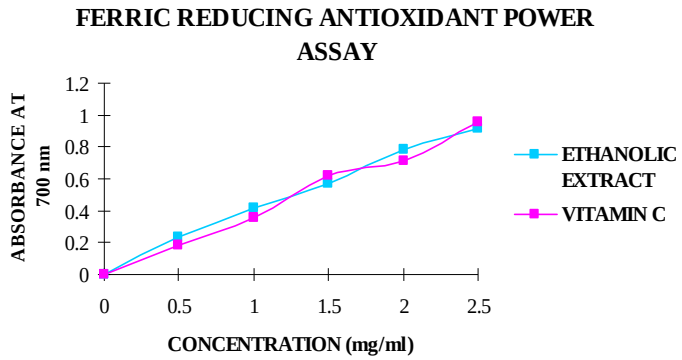

Fig. 7: Ferric reducing antioxidant power assay.

Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action[23]. In the reducing power assay, the presence of antioxidants in the samples would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability. Figure 8 shows dose-response curves for the reducing powers of the extract. The reducing power ability of the ethanolic extract was found to be 0.86 mg/ml, compared with standard vitamin C 0.89 mg/ml respectively. It was found that the reducing power of the extract increased with the increase of their concentrations.

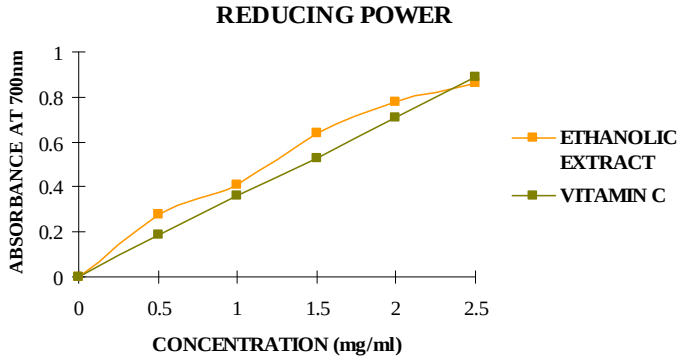

Fig. 8: Reducing power assay.

Total antioxidant capacity of the *Ananus comosus* extract, expressed as the number of gram equivalents of ascorbic acid, is shown in figure 9. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm[24]. The total antioxidant capacity of the ethanolic extract was found to be 435.91 nmol/g ascorbic acid. The above results were corroborate with the study of Sathish Kumar et al. [25] in *Canthium parviflorum.*

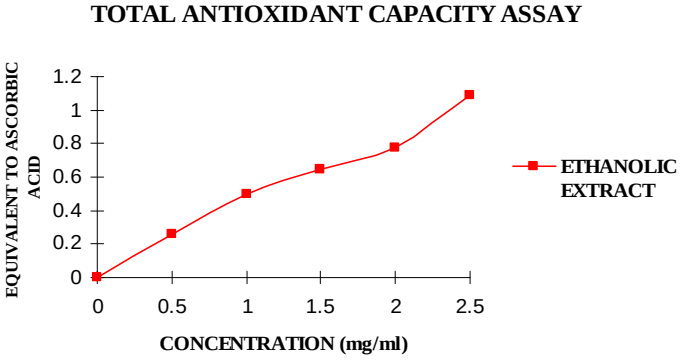

Fig. 9: Total antioxidant capacity assay.

In the process of the thiobarbituric acid (TBA) method, the formation of malonaldehyde is the basis for evaluating the extent of lipid peroxidation. The conditions of low pH and high temperature (100°C), malonaldehyde could bind TBA to form a red complex which could be determined at 532 nm. The increase in amount of red pigment formed correlates with the oxidative rancidity of the lipid[26].

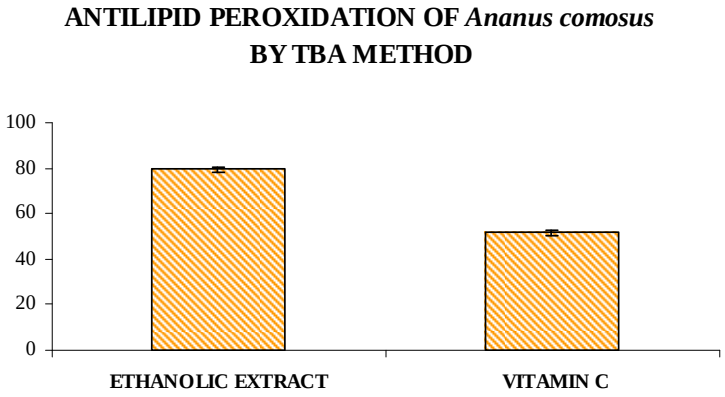

Fig. 10: Anti-lipid peroxidation activity of *Ananus comosus* peel.

Anti-lipid peroxidation of *Ananus comosus* extract were shown in figure 10. The activity of the assay was found to be 79.34% when compared with standard antioxidant Vitamin C (51.72%) at a concentration of 5mg/ml. Hence the ethanolic extract shows higher antioxidant activity than that of vitamin C.

CONCLUSION

In conclusion, the results presented in this report indicated that ethanolic extract of *Ananus comosus* peel efficiently attenuated oxidative stress via its antioxidant properties. However, further studies are needed to isolate active principles responsible for the overall antioxidant activity of the extract.

ACKNOWLEDGEMENT

We express our deep sense of gratitude to Karpagam University management for its support and motivation.

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