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TO EVALUATE *IN VITRO* ANTIOXIDANT PROPERTY OF SUGARCANE *(SACCHARUM OFFICINARUM* L) PEEL

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ABSTRCAT

The Sugarcane (Saccharum officinarum L.) is a widely distributed plant. It is an important source for sugar and raw material for alcohol production. In the present study, sugarcane peel was used as a source for anthocyanin extraction. Anthocyanins are natural pigments, which are responsible for red, purple and blue colours in parts of the plant. It belongs to a major flavonoid class which has strong antioxidant activity. In the present scenario, there is a rising demand for natural sources of food colorants with nutraceutical benefits. So the present research focus on the antioxidant activity of anthocyanin extracted from sugarcane peel. Antioxidant assays were analysed by DPPH, hydroxy radical scavenging, reducing power, metal chelating, hydrogen peroxide, deoxyribose degradation (site specific & non -site specific) and ABTS assays. From the results, it was confirmed that anthocyanin extracted from sugarcane peel has potent antioxidant properties. Finally, ensure that the anthocyanin extracted from sugarcane peel replace many synthetic food colorants and chemical antioxidants.

KEY WORDS : Sugarcane peel, anthocyanin, antioxidants, DPPH, hydrogen peroxide.

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INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is a source not only for sugar and raw material for alcohol production, but also for a variety of other products. Sugarcane extracts often contain potent polyphenolic antioxidants such as anthocyanin and tannins (Wang *et al*., 1997). Pigments present in sugarcane juice are mainly phenolic compounds. Paton & Duong, 1992 reported the phenolic composition of sugarcane and its products. Such compounds are mainly phenyl propanoids and flavonoids, major representatives of the latter being derivatives of naringenin, Tricin, apigenin and luteolin (Smith & Paton 1986; Williams, Harborne &Clifford 1974). Polyphenols exhibit many biological properties such as antiinflammatory, antiallergic, antibacterial, cardio protective and antioxidant activities. Phenolic substances in sugarcane may exert biological captivities (Duarte- almeida, Vidal novoa, Fallarero linares, Lajola & Genvese, 2006) and Antiproliferative activity in sugarcane (Duarte–almeida, negri, salatino, carvalho & Lajola, 2007.

Anthocyanins are polyphenolic compounds responsible for cyanic colors ranging from salmon pink through red and violet to dark blue of most flowers, fruits, leaves and stems. They comprise the largest group of the water soluble pigments in the plants kingdom (Strack & Wray, 1994). Anthocyanin pigments have intensified because of their health benefits as dietary antioxidants, cancer prevention and coronary heart disease. Anthocyanins are one class of flavonoids which are widely distributed plant Polyphenols. They attract pollinators, seed dispersers; protect plant tissue from proto inhibition and oxidation resulting from photosynthesis (Gould & Lee, 2002). Flavonoids, flavon-3-ols, flavones, flavanones and flavanonols are additional classes of flavonoids that differ in their oxidation state from the anthocyanin. (Ronald E.wrolstad). Recent studies have shown that many flavonoids and related Polyphenols contribute significantly to the total antioxidant activity of many fruits and vegetable (Luo, Basile & Kenelly 2002; Vinson *et al*., 1999). Only very recently physiological effects of four types of extracts were described by Japanese researchers (Nagai *et al*., 2001; Koge *et al.,* 2002) viz., promotion of resistance against viral and bacteria infections, stimulation of immune response, protection against liver injuries, free radical scavenging activity and growth in chickens. It is estimated that human consume between a few 100mg to 1g flavonoids every day (Halloman and Katan 1999; Pieta 2000). Human studies have found the flavonoids appear in blood plasma, at pharmacologically active levels, after eating certain foods but do not accumulate in the plasma (Cao, Booth, Sadowski & Prior 1998; Holloman & Katan 1999). Cyanidine is the most common anthocyanin which has potential antioxidant effects (Wang *et al*., 1997). Glycosylation and hydroxylation of the anthocyanin backbone affects antioxidant activity (Wang et., al 1997). Anthocyanin glycosides remain intact when passing from the digestive tract into the blood circulation of mammals (Miyazawa, Nakagawa, Kudo, Muraishi& someya 1999).

METHODS AND MATERIALS

Antioxidant assays

Scavenging activity of DPPH radical

Scavenging activity of anthocycanins against DPPH radicals was assessed according to the method of Larrauri, Sanchez-Moreno, and Saura Calixto (1998) with some modifications. Briefly, 0.1 mM DPPH-methanol solution was mixed with 1 ml of 0.1mM DPPH methanol solution. After the solution was incubated for 30 min at 25º C in dark, the decrease in the absorbance at 517nm was measured. Control contained methanol instead of antioxidant solution while blanks contained methanol instead of DPPH solution in the experiment. Ascorbic acid and BHT were used as positive controls. The inhibition of DPPH radicals by the samples was calculated according to the following equation:

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DPPH-scavenging activity (%) = [1-(absorbance of the sample-absorbance of blank)/absorbance of the control] ×100*Hydroxyl radical scavenging activity*

The hydroxyl radical scavenging activity was determined according to the methods described by Singh, Murthy and Jayaprakash (2002). 0.1 ml of the different extracts of anthocyanin samples extract was taken in different test tubes. 1.0 ml of iron-EDTA solution (0.1% ferrous ammonium sulfate and0.26% EDTA), 0.5 ml of DMSO (0.85% v/v in 0.1 M Phosphate buffer, p^H 7.4) were added to these tubes, and the reaction was initiated by adding 0.5 ml 0f 0.22% ascorbic acid. Test tubes were capped tightly and heated on a water bath at 80◦C - 90◦C for 15 min. The reaction was terminated by the addition of 1 ml of ice cold TCA (17.5 %w/v). 3 ml of Nash reagent (75 g of ammonium acetate, 3 ml of glacial acetic acid, and 2 ml of acetyl acetone were mixed and raised to 1 L with distilled water) was added to all of the tubes and left at room temperature for 15 min for the color development. The intensity of the yellow color formed was measured spectrophotometrically at 412 nm against the reagent blank. The percentage of hydroxyl radical scavenging activity is calculated by using the formula:

% of hydroxyl radical scavenging activity=1-absorbanceof sample/absorbance of blank×100

Determination of reducing power

The reducing power was determined according to the method of Oyaizu (1986). A 0.25ml aliquot of various concentrations of anthocyanins was mixed with 2.5 ml of 200mM sodium phosphate buffer (p^H 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50 ºC for 20 min. After 2.5 ml of 10% trichloroacetic acid (w/v) were added, the mixture was centrifuged at 650g for 10 min. A 5ml aliquot of the upper layer was mixed with 5ml of distilled water and 1ml of 0.1% ferric chloride at 700nm was measured. A higher absorbance, higher the reducing power.

Metal chelating activity

The chelation of ferrous ions by the extract was estimated by the method of Dinis et. al. (1994) with slight modification and compared with that EDTA, BHT and that of ascorbic acid. The chelation test initially includes the addition of ferrous chloride. The antioxidants present in the samples chelates the ferrous ions from the ferrous chloride. The remaining ferrous combine with ferrozine to form ferrous ferrozine complex. The intensity of the ferrous ferrozine complex formation depends on the chelating capacity of the sample and the colour formation was measured at 562 nm (Shimadzu UV-Vis 2450).

Different concentrations of standard and extracts (100-500 µg/ml) were added to a solution of 100 µg $FeCl_2$ (1mM).The reaction was initiated by the addition of 250 µl ferrozine (1 mM). The mixture was finally quantified to 1.3 ml with methanol, shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was measured spectrophotometrically. All the test and analysis were done in duplicate and average values were taken. The percentage inhibition of ferrous-ferrozine complex formation was calculated using the formula;

%= 1-As/Ac X 100. Where, 'Ac' is the absorbance of the control, 'As' is the absorbance of the sample.

Determination of inhibitory effect on deoxyribose degradation

Inhibitory effect of the anthocyanin on deoxyribose degradation was determined by measuring the reaction activity between either antioxidants or hydroxyl radicals (referred to as non-site specific scavenging assay) or antioxidants and iron ions (referred to as site specific scavenging assay) described by Lee *et. al.,* (2002). For non-site specific scavenging assay, 0.1 ml aliquot of different concentration of anthocyanin was mixed with 1ml of reaction buffer (100 µM $FeCl_3$, 104 µM EDTA, 1.5mM H_2O_2, 2.5mM deoxyribose, and 100µM L-Ascorbic acid, p^H

7.4) and incubated for 1 hour at 37^0 C. A 1ml aliquot of 0.5% 2-thiobarbituric acid in 0.025 M NaOH and 1 ml of 2.8% trichloroacetic acid were added to the mixture and it was heated for 30 min at 80^0 C. The mixture was cooled on ice and the absorbance was measured at 532nm. Site specific activity, which represented the ability of anthocyanin to chelate iron ions and interfere with hydroxyl radical generation, was measured using the same reaction buffer without EDTA. Percent inhibition of deoxyribose degradation was calculated as

% = (1-absobance of sample/absorbance of control) ×100. Control without sample.

. H_2O_2 Scavenging Activity

Hydrogen Peroxide scavenging activity assay is common method of antioxidant activity experiments. 100µl of sample with 1.5ml of phosphate buffer p^H 7.4 and 0.6ml of H_2O_2 . The readings were measured spectrophotometrically at 230 nm. The H_2O_2 scavenging activity is calculated by percentage,

$$\% = A_o - A_1 / A_o \times 100.$$

Where A_o = Absorbance of Control
A_1 = Absorbance of samples

ABTS Assay

The free radical scavenging activity of sugarcane was determined by ABTS radical cation decolourization assay (Re *et al*.,1999). ABTS was dissolved in water to a 7mM concentration. ABTS radical cation ($ABTS^{\cdot +}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulphate (Final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 hours before use. Because ABTS and potassium persulphate react stoichiometrically at a ratio of 1:0.5 (mol/mol), this will result in incomplete oxidation of the ABTS. Oxidation of the ABTS commenced immediately, but the absorbance was not maximal and stable until more than 6 hour had elapsed. The radical was stable in this form for more than 2 days when stored in the dark at room temperature (Katalinic, Milos, Kulisic and Jukic 2005) prior to use, the stock solution was diluted with ethanol (1:89 v/v) to an absorbance of 0.70($\pm$0.02) at 734 nm and equilibrated at 30°C exactly 6 mins after initial mixing (A_t). One mililitre (1ml) of diluted ABTS solution was mixed with 10 µl of extract of different strength (4-8 mg/ml). the percentage decrease of absorbance at 734 nm was calculated for each point and the antioxidant capacity of the rest compounds was expressed percent inhibition (%I) and I_c 50 value was calculated from regression analysis. Quercetin (2-10 µM/ml) was used as a standard antioxidant.

Total antioxidant assay

The antioxidant assay of Anthocyanin against lipid peroxidation was measured through ammonium thiocyanate assay as described by Takao, Kitatani, Watanable, Yagi and Sakata (1994) with some modifications.The reaction solution containing 0.2ml of 1, 10, 50mg/ml Anthocyanin extract, 0.2ml of linoleic acid emulsion (25mg/ml in 99% ethanol) and 0.4ml of 50mM phosphate buffer (pH 7.4) was incubated in the dark at 40ºC. A 0.1 ml of 3ml of 70 %(v/v) ethanol and 0.1ml of 30%(w/v) ammonium thiocyanate. Presently 3 minute after the addition of 0.1ml of 20mM ferrous chloride in 3.5%(V/V) hrdrochloric acid to the reaction mixture ,the absorbance of the resulting red color was measured at 500nm. Aliquots were assayed every 24 hour until the day after the absorbance of the control solution (without Anthocyanin extract) reached maximum value. Ascorbic acid and BHT were used as positive control.

RESULTS AND DISCUSSION

ANTIOXIDANT ACTIVITY ASSAYS
SCAVENGING ACTIVITY OF DPPH RADICAL ASSAY

The DPPH activity is a measure of the reactivity decreases in absorbance at 517nm.As the reaction between DPPH and antioxidant progressive. Huang *et al*., and

Singh & Ragini (2004). The ability of phenolic compound quench reactive species by hydrogen donation was measured through the DPPH radical scavenging activity assay. Antioxidant activity was evolved with percentage inhibition values, the concentration and the radical scavenging activity as listed in the Table 1& Figure 1. The result indicated the percentage of inhibition values of anthocyanin from sugarcane peel extract ranges from 43% to 60% at a concentration ranging from 1mg to 100mg. The high radical scavenging property of *M.esculenta* may be due to the hydroxyl groups existing in the phenolic compounds chemical structure that can provide the necessary component as a radical scavenger Tapan *et.al*. (2011).

HYDROXYL RADICAL SCAVENGING ACTIVITY ASSAY

The hydroxyl radical is extremely reactive free radicals found in biological systems and has been implicated as higher damaging species in free radical pathology. Capable of damaging almost every molecule found in living cells. This species is considered to be one of the quick initiator of lipid peroxidation process, abstracting hydrogen atom from unsaturated fatty acids (Kappus, 1991). The hydroxyl radical scavenging activity of sugarcane peel was given in Table 1& Figure 2. The hydroxyl radical scavenging activity of the sugarcane peel extract exhibited a highest activity of 73.2% at a concentration of 100mg/ml. The ability of anthocyanin to quench hydroxyl radical seems to be directly related in prevention of propagation of the lipid peroxidation. Nataraj *et al*., 2010 reported maximum hydroxyl radical scavenging activity in *M.alba* at a concentration of 3µg/ml.

REDUCING POWER

The reducing power was determined by according to method of Oyaizu in the year 1986. The potassium ferric cyanide reduction method was used to measure the ability of phenolic compound to quench radicals through electron donation. The activity of total antioxidant of sugarcane peel anthocyanin is measured by spectrophotometer and in which the change in absorbance is measured when the antioxidant reduced ferric ion- cyanide complex to the ferrous form and resulted with higher absorbance value (Chou *et al*., 2003) in Table 1 & Figure3. shows reducing power of the different concentrations of sugarcane peel anthocyanin. In the present study, the highest reducing power was absorbed in 100mg/ml. Polyphenolics in the anthocyanin extracts appear to function as good electron and hydrogen atoms donor and therefore should be able to terminate radical chain reaction by converting free radicals to more stable products.

METAL CHELATING ACTIVITY

Ability of antioxidants to form insoluble metal complexes with ferrous ion or to generate stearic hindrance that prevent interaction between metal and lipid is evaluated using the ion chelating capacity (Hsu *et al*., 2003). The activity is measured by monitoring the decrease in absorbance of lead ferric-ferrozine complex as antioxidants compete with ferrozin in chelating ferrous ion (Elmastas *et al*., 2003) Table 1 &Figure 4 shows the metal chelating power of different concentrations of sugarcane peel anthocyanin. From the table, it is clear that chelating power of anthocyanin extract increase with increase in concentration. This may indicate the presence of antioxidant responsible for metal chelating. Lee *et al*., in the year 2004 reported that non-phenolic metal chelators includes phosphoric acid, carnosin, some amino acids, peptide and proteins such as transferring, ovo-transferrin are responsible for metal chelation.. Since in our extracts there is no nonphenolic metal chelators which are responsible for metal chelation. Of the metal chelating activity may be due to presence of phenolic compounds present in sugarcane peel extract.

HYDROGEN PEROXIDE SCAVENGING ACTIVITY

Hydrogen Peroxide may be generated *in vivo* by several oxidase enzymes or by activated phagocytosis during killing of

bacterial and fungal strains. There is increasing evidence that hydrogen peroxide either directly or indirectly via its reduction product, $OH^{\cdot}$ radical may be acts as a messenger molecule in the synthesis and activation of several inflammatory mediators. Table 1&Figure 5 and showed a capacity of hydrogen peroxide scavenging activity.83% inhibition was observed in sugarcane peel anthocyanin at concentration of 1mg/ml. Zimenz *et al*., 2008 reported that hydrogen peroxide scavenging activity is due to the high content of phenolic compounds in Apricot samples. Radi *et al*., 1997 and Chun *et al*., 2003 suggest that the hydrogen peroxide scavenging activity occurs due to enzyme polyphenol oxidases which are localized in plastids, and phenolic compounds are mainly located in the vacuole, when the subcellular compartmentalization is lost during homogenization.

INHIBITORY EFFECTS OF DEOXYRIBOSE DEGRADATION

$OH^{\cdot}$ radical can be formed by Fenton reaction in the presence of reduced transition metals such as Fe^{2+} and hydrogen peroxide which is known to be the most reactive of all the reduced form of dioxygen and it starts to cell damage *in vivo* (Rollett-Labelle *et al*., 1998) to determine whether sugarcane peel anthocyanin reduced hydroxyl radical generations by chelating metal ions or by directly scavenging hydroxyl radicals. The effects on anthocyanin or hydroxyl radical generated by Fe^{3+} ions were analyzed by determining the degree of deoxyribose degradations. Table 1 & Figure 6 shows the concentrations.

ABTS ASSAY

The ABTS scavenging activity increases with increase in the amount of anthocyanin. Therefore it appears the ability of anthocyanin to Scavenge the ABTS radical cation increases on extension of the chromophore. The presence of functional group in anthocyanin modulates the radical scavenging activity the incorporation of carboxyl groups in the anthocyanin ring of the profound suppressive effect of the ABTS radical scavenging effect Declos *et al.,* 1996 reported the presence of OH groups at the carbon ring does not reduce the carotenoid ability to scavenge ABTS radical activity.

TOTAL ANTIOXIDANT ASSAY

In present study, the antioxidant activity of anthocyanin was determined by peroxidation of linoleic acid using ferric thiocyanate method. During linoleic acid peroxidation , peroxides were formed and these compounds oxidized Fe^{2+} to Fe^{3+}. The Fe^{3+} ion forms a complex with SCN^{-} , which had a maximum absorbance at 500 nm(Takao.*et.al.,*1994). Thus a high absorbance value was an indication of high peroxide formation during the emulsion incubation as shown in Fig.8 inhibit peroxidation of linoleic acid and reduce formation of hydrogen peroxide. Thus anthocyanin are These results indicate that anthocyanin can significantly powerful natural antioxidant.

CONCLUSION

Sugarcane (*Saccharum officinarum*) peel extract was shown to contain a range of phenolic molecules like flavonoids and anthocyanin. The result of present study showed that the extract of sugarcane peel, which contain highest amount of phenolic compounds exhibited the greatest radical scavenging activity and reducing power. The radical scavenging activities of the sugarcane peel extracts was found to be effective than the commercial available synthetic like BHT. As the plant extracts were quit safe and the use of synthetic antioxidant was limited because of their toxicity, therefore this sugarcane peel could be exploited as antioxidant additives. Altogether, current results support the notion that natural phenolics antioxidants present in sugarcane peel extract could be a useful alternative theraphy for relative oxidative stress.

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