Abstract

The present study investigated the antioxidant and phenolic composition of sugarcane. The leaves and juices of thirteen varieties of sugarcane were studied for their antioxidant activity and protective effect on DNA damage. 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) assay was used to determine the radical scavenging activities in leaves and juices. Different varieties of sugarcane showed good antioxidant properties, IC50 values ranged from 20.82 to 27.47 µg/ml for leaves and from 63.95 to higher than 200 µg/ml for juice. The leaves and juice possess strong ability to protect against DNA damage induced by hydroxyl radical generated in Fenton reaction. The major phenolic acids, some flavonoid aglycone and glycosides were identified in leaves by high performance liquid chromatography. Ferulic acid (14.63±0.03 mg/g), cumaric acid (11.65±0.03 mg/g), quercetrin (10.96±0.02 mg/g), caffeic acid (9.16±0.01 mg/g) and ellagic acid (9.03±0.02 mg/g) were predominant in infusion of sugarcane.
Phenolic profile, antioxidant potential and DNA damage protecting activity of sugarcane (Saccharum officinarum)

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Article info
Article history:
Received 8 July 2013
Received in revised form 16 September 2013
Accepted 19 September 2013
Available online 29 September 2013

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1. Introduction
In the last several decades, there has been a huge interest in identifying free radical scavengers or antioxidants that can inhibit or retard the oxidative DNA damage. These antioxidants supplied by diets, include vitamin C, vitamin E, carotenoids (β-carotene, α-carotene, β-cryptoxanthin, lutein, zeaxanthin and lycopene) to several polyphenolic compounds including flavo noids (catechins, flavonols, flavones and isoflavonoids) can impede carcinogenesis by scavenging free radicals or interfering with the binding of carcinogens to DNA (Craig, 1997; Stoner & Mukhtar, 1995). Accordingly, antioxidants, abundant in foods, have received the great attention and have been studied extensively, since they can reduce risks for cardiovascular disease or several types of cancers (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993; Kromhout et al., 1995).

Sugarcane is an important cash crop of Pakistan. It is mainly grown for sugar production. It is an important source of income and employment for the farming community of the country. It also forms essential item for industries like sugar, chip board, paper, barages, confectionery, uses in chemicals, plastics, paints, synthetics, fiber, insecticides and detergents (Aslam & Khan, 2001). Sugarcane, popularly known as noble cane, due to its high sucrose content and low fiber content is one of the important industrial crops of the world. The sugarcane juice contains flavonoids such as apigenin, luteolin and tricin derivatives and among phenolics, hydroxycinnamic, caffeic and sinapic acid, representing a total content of around 160 mg/l (Joaquim, Mauricio, Adyary, Franco, & Maria, 2006) whereas sugarcane leaves contains luteolin-8-C-(rhamnosylglucoside) as major compound with radical scavenging activity (Fabiana, Renata, Tatiana, & Janete, 2008). It is principal raw material for the sugar industry as 70% of the world's sugar comes from sugarcane. When the sugar is produced, the sugarcane leaf is removed prior to a series of manufacturing procedures. The leaves are by products of agro-industries and are wasted as feedstock and fertilizer. Sugarcane leaves are commonly discarded or burned. However, the disposal has become a matter of serious concern. Hence, more rational uses of sugarcane leaves are necessary. Besides sugar production, large number of population in the tropics and sub tropics relishes its juice and consume raw cane. In the Pakistan chewing raw sugarcane is recommended for sound and healthy body. Both the roots and stems of sugarcane are used in medicine to treat skin and urinary tract infections, as well as for bronchitis, heart conditions, loss of milk production, cough, anemia, constipation as well as general debility. It is also used for jaundice and lowering blood pressure (Miza et al., 2011). The DPPH radical assay has been widely used to test the ability of compounds
as free-radical scavengers or hydrogen donors and to evaluate the antioxidant activity of plant extracts and foods (Forti, Caligaris, Celioli, & Nicoli, 2000; Sewo, Amnu, & Chandran, 2012). In view of the potential role of sugarcane as a dietary source of flavonoids as well as its possible use as functional food, this study was aimed to evaluate the antioxidant activity of thirteen varieties of sugarcane, their phenolic and flavonoid contents and to examine their protective effect on hydroxyl radical mediated DNA damage. To our knowledge this is first report on the antioxidant and DNA protecting ability of these sugarcane cultivars.

2. Material and methods

2.1. Chemicals and reagents

2.2-Diphenyl-1-picrylhydrazyl radical (DPPH) Sigma, 90.0%), 1,1,1-tris (hydroxymethyl) ethane (Tris) and Low melting agarose were purchased from Sigma and Aldrich Chemicals (St, Louis, MO, USA); Ethanol (50%), iron sulphate, phosphate buffer, hydroxyl peroxide, sodium acetate and sodium carbonate were purchased from Bio-Chemical (Labore). Methanol, acetic acid, gallic acid, chlorogenic acid, caffeic acid, ferulic acid, cumaric acid and ellagic was purchased from Merck (Darmstadt, Germany). Quercetin, quercitin, isoquercetin, rutin, catechin and epicatechin were acquired from Sigma Chemical Co. (St, Louis, MO, USA). All the chemicals and reagents were of analytical grade.

2.2. Sugarcane samples

Studies were conducted on thirteen genotypes of sugarcane which were collected from different growing areas of Pakistan. Genotypes selected for experiments were NSG-45, CPF-198, Uho 83-153, CPT-246, CSSG-668, S-2003-US-718, RB-72, S-2002-US-160, RO-1148, NSG-555, S-2003-US-694, S-2003-US-633 and CPT-237. These genotypes were chosen for their frequent cultivation in the area and use by local communities. All genotypes were grown under glass house conditions in Faculty of agriculture, University of Poonch Rawalakot Pakistan. Mature sugarcane leaves (4 months old) yellowish green in color were harvested from aerial parts of plants. Three samples of leaves were chosen from each plant.

2.3. Preparation of sugarcane extracts

The leaves were air dried in order to remove the moisture. The leaves of sugarcane (5 g) were ground and soaked in boiling water (250 ml) for 15 min, allowed to cool and filtered using Whatman filter paper. The highly significant total phenolic content and antioxidant activity has been recorded in plant sample extract with distilled boiling water (Panamani, Sathishkumar, & Lakshmi, 2011). The obtained residue was further extracted twice and finally the whole extract was concentrated. The extract weight and percentage yield were found to be 0.5–1.5%, respectively. The serial dilution of the extract was made to obtain the desired concentration of plant for experiment.

Sugarcane juice was extracted from the genotypes and filtered to obtain the juices.

2.4. Antioxidant activity by DPPH radical scavenging

The antioxidant activities of the sugarcane extracts were measured using the stable DPPH radical according to the method of Hatano, Kagawa, Yashuka, and Okuda (1998). Briefly 0.25 mM solution of DPPH radical (0.5 ml) was added to the sample solution in ethanol (1 ml) at different concentrations (25–300 µg/ml) of aqueous extract of sugarcane. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was measured at 517 nm. The capacity to scavenge the DPPH radical was calculated using the following equation: [% DPPH Scavenging (%) = 100; Where, A0 is the absorbance of the control reaction and A1 is the absorbance of the sample itself]. The IC50 values (Extract concentration that cause 50% scavenging) were determined from the graph of scavenging effect percentage against the extract concentration. All determinations were carried out in triplicate.

2.5. Total antioxidant activity assay

The assay was based on the reduction of molybdenum. Mo(VII)-Mo(V) by the extract and subsequent formation of a green phosphomolybdate complex at acidic pH (Prieto, Prieto, & Alvar, 1999). The extract (0.1 mg/ml) was mixed with 3 ml of the reagent solution (0.5 M H2SO4, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95 °C for 60 min. The reaction was cooled to room temperature and the absorbance of the solution was measured at 820 nm.

2.6. DNA extraction

DNA was extracted from leaves of sugarcane with new modified method (Li & Midmore, 1999). DNA of all sample were stored at -20 °C. The presence of DNA was confirmed by gel electrophoresis (1.5%).

2.7. Site-specific hydroxyl radical–mediated DNA strand breaks

The site specific hydroxyl radical–mediated DNA strand breaks were measured by the procedure described by Yeung et al. (2002) with some minor modifications. Briefly, 0.5 µg DNA was incubated with 1 µl of 1 mM FeSO4, 1 µl of 10% H2O2, 3 µl of 100 µg/ml of aqueous extracts of sugarcane, and the final volume was made up to 15 µl with 50 mM phosphate buffer (pH 7.0). The mixture was incubated in water bath at 37 °C for 30 min. After the incubation, the sample was immediately loaded in a 1.5% agarose gel along with 3 µl ethidium bromide, containing 40 mM Tris, 20 mM sodium acetate and 1 mM EDTA, and electrophoresed in a horizontal slab apparatus in Tris/boric/EDTA gel buffer. The gel was then photographed under UV light.

2.8. HPLC–DAD- analysis of phenolic compounds

High performance liquid chromatography (HPLC–DAD) was performed with a Shimadzu Prominence Auto Sampler (SH-20A) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A diode array detector and LC solution 1.22 SPF software. Reverse phase chromatographic analyses were carried out under gradient conditions using C8 column (4.6 mm × 250 mm) packed with 5 µm diameter particles; the mobile phase was water containing 2% acetic acid (A) and methanol (B), and the composition gradient was: 5% (B) for 2 min; 25% (B) until 10 min; 40%; 50%; 70% and 80% (B) every 10 min; following the method described by Bologn et al. (2012) with slight modifications. Saccarinum officinarum (sugarcane) infus leaves and mobile phase were filtered through 0.45 µm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use, the extracts of sugarcane were analyzed at a concentration of 30 mg/ml. The flow rate was 0.7 ml/min and the injection volume was 50 µl. The sample and mobile phase were filtered through 0.45 µm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration range.
of 0.025–0.300 mg/ml catechin, epicatechin, quercetin, quercitrin, isoquercitrin and rutin, and 0.045–0.500 mg/ml for gallic, chlorogenic, caffeic, ferulic, cumaric and ellagic acids. Quantification was carried out by integration of the peaks using the external standard method, at 257 nm for gallic acid, 280 nm for catechin and epicatechin, 325 nm for ellagic acid, chlorogenic acid, caffeic acid, ferulic acid and cumaric acid, and 365 for quercetin, quercitrin, isoquercitrin and rutin. The chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200–600 nm). All chromatography operations were carried out at ambient temperature and in triplicate.

2.9. Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were calculated based on the standard deviation of the responses and the slope using three independent analytical curves, as defined by Boligon et al. (2013). LOD and LOQ were calculated as 3.3 and 10 σS, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve.

2.10. Statistical analysis

The results were expressed as means ± standard deviation. The data was analyzed by one way ANOVA and different group means were compared by Duncan's multiple range (DMR) test where necessary. P < 0.05 was considered significant in all cases. The software Package Statistica was used for analysis of data.

3. Results and discussion

3.1. DPPH radical scavenging activity

Free radicals involved in the process of lipid peroxidation are considered to play a major role in numerous chronic pathologies such as cancer and cardiovascular diseases (Halliwell, Gutteridge, & Cross, 1992). DPPH is considered to be a model of a stable lipophilic radical. A chain reaction of lipophilic radicals is initiated by lipid autoxidation. Antioxidants react with DPPH*, reducing the number of DPPH free radical to the number of their available hydroxyl groups. Therefore the absorption at 517 nm is proportional

![Graph](https://www.researchgate.net/publication/258425639_Phenolic_profile...)

**Fig. 1. Antioxidant activity of aqueous extract of sugarcane** (a) DPPH radical scavenging activity of different genotypes of sugarcane leaves. (b) DPPH radical scavenging activity of different genotypes of sugarcane juice. Values are mean ± SD (n = 3).
to the amount of residual DPPH. It is visually noticeable as a
discoloration from purple to yellow. Among different genotypes
NSG-555, S-2003-US-633, CPF-237 and CPF-246 significantly dis-
played higher DPPH radical scavenging activities in leaves (Fig
1a) whereas NSG-45, CPF-198, CSSG-668, S-2003-US-694 also
showed good antioxidant activities in juice (Fig 1a and b). It is
interesting that the aqueous extracts of these genotypes displayed
antioxidant activity higher than 50% even at a low concentration of
25 μg/mL. The extract concentration that cause 50% scavenging of
DPPH (IC50 value) are shown in Table 1. The IC50 is inversely pro-
tional to the scavenging activity of the extract. The IC50 values
of different genotypes of sugarcane varied from 20.82 to
27.47 μg/mL which indicates the high radical scavenging activity
of sugarcane. The extracts from the leaves of different genotypes,
CPF-246 and NSG-555 demonstrated the highest antioxidant ac-
activity (IC50 20.82 μg/mL) whereas, Uho 84-153 relatively showed less
antioxidant activity (IC50 27.47 μg/mL) NSG-45, NSG-555, CPF-
198, S-2003-US-633 displayed similar IC50 (Table 1). In comparison
to the leaves, juice displayed lesser antioxidant activity (IC50 val-
ues in the range of 63.95-200 μg/mL). NSG-55 displayed the high-
est antioxidant activity (IC50 63.95 μg/mL) (Table 1). The IC50
values of S-2003-US-718 and RB-72 could not be calculated as it
was higher than 200 μg/mL (Table 1).

The results obtained in this study have clearly indicated the high
antioxidant activity of sugarcane cultivars and suggest their use
in diseases arising from free radicals. The antioxidant activity
of sugarcane juice is also supported by the literature (Fabiana
et al., 2008; Jaouqim et al. 2006; Kadam et al., 2008). The anti-
oxidant activity of aqueous extract sugarcane leaves is supported by
the studies of Pei-Ying Yu (2009) where three varieties of sugar-
cane displayed significant total antioxidant activity, reducing
activity and liposome peroxidation inhibition studies.

3.2. Total antioxidant activity

In the phosphomolybdenum assay, which is a quantitative
method to evaluate water-soluble and fat-soluble antioxidant
capacity (total antioxidant capacity), the extracts demonstrated
electron-donating capacity showing its ability to act as chain ter-
minators, transforming relative free radical species into more sta-
ble non-reactive products (Dorman, Kosar, Kahlos, Holm, &
Hiltunen, 2003). Ahlhorn, Klapty, and Akerstrom (2005) reported
that the reducing property can be a novel antioxidant defense
mechanism, possibly through the ability of the antioxidant com-
 pound to reduce transition metals. Therefore, the higher reducing
ability of the sugarcane extracts may have contributed to the high-
er antioxidant activity. The total antioxidant activity of the extract
(equivalent to ascorbic acid) ranged between 62.3 and 80.26 μg
mL CPF-246 was found to be the potential candidate of antioxidant
activity (80.26 μg/mL) whereas, Co-1148 showed the least antioxi-
and CPF-198 demonstrated the similar antioxidant activities (Fig
2a). In comparison to the leaves juices showed higher reducing
activity as their antioxidant activity varied between 70.43 and
99.9 μg/mL. Co-1148 and CPF-246 displayed the highest reducing
activities (Fig 2b). The high reducing activity of juice may be due to
the high content of ascorbic acid which is less in leaves.

3.3. Protection against DNA damage

In order to study the protective effects of sugarcane aqueous ex-
tract on hydroxyl radical-mediated DNA strand breaks, three
genotypes of sugarcane were used in the site specific DNA damage
assay. Incubation of plant DNA with FeSO4 and H2O2 for 40 min in
water bath resulted in producing hydroxyl ions, whereby indicat-
ing that both sin gle-strand and double-strand DNA breaks can
be induced by FeSO4/H2O2 at the indicated concentrations and
incubation time (Fig 3). The gel pattern of DNA exposed to
FeSO4 + H2O2 in the presence and the absence of extracts of sugar-
cane is presented in Fig 3. The control showed the absence of spe-
cific band in treated DNA (shredding) which indicates DNA
damage. Different genotypes of sugarcane extracts at a final con-
centration of 20 μg/mL significantly reduced the DNA damage
(Fig 3). Hydroxyl radicals generated by the Fenton reaction are
known to cause oxidatively induced breaks in DNA strands to yield
its fragmented forms. The free radical scavenging activity of sugar-
cane was studied on genomic DNA. The treatment of supercoiled
DNA with Fenton’s reagent directed the alteration of DNA to open
 circular form. The addition of extracts to the reaction mixture sub-
stantially decreased the DNA strand scission and retained the
supercoiled form, thus effectively protects DNA even at a low con-
centration of 20 μg/mL. The genotypes showed their ability to pro-
tect against DNA damage induced by hydroxyl radicals. These
results are in line with our previous studies (Abbas et al., 2013).
Based on the previous data, it is possible that the powerful anti-
oxidant activity of sugarcane aqueous extracts is given by the pres-
ence of substances with hydroxyls. In this context, flavonoids
possess an ideal structure for the scavenging of free radicals since
they present a number of hydroxyls acting as hydrogen donators
acting as an important antioxidant agent (Cao, Sofic, & Prior,
1997). In fact, polyphenols are an important group of pharmaco-
logically active compounds. They are considered to be the most ac-
tive antioxidant derivatives in plants (Edenharder & Gunhage,
2003). However, it has been shown that the phenolic content does
not necessary follow the antioxidant activity. Antioxidant activity
is generally the result of the combined activity of a wide range of
compounds, including phenolics, peptides, organic acids and other
components (Gallardo, Jimenez, & Garcia-Conesa, 2006). This is
first report to date on the protective effect of sugarcane leaves on
DNA damage. Kadam et al. (2008) reported that sugarcane juice
has protective role against radiation induced DNA damage. Sugar-
cane extracts and juices are able to exhibit the protection on DNA
damage caused by hydroxyl radical, which might be due to their
chelating activity on iron or hydroxyl radical scavenging or both
(Sabir et al., 2012).

3.4. Content of phenolics, flavonoids and HPLC characterization of phenolic compounds

The HPLC phenolic profile of S. officinarum in infusion of leaves
was also acquired. HPLC analysis is shown in Fig. 4. The samples
of S. officinarum (CPF-246) displayed the highest antioxidant activity,
therefore, its phenolic profile was determined. The results revealed
that the infusion contains several compounds in addition to gallic
acid (retention time-tR 10.97 min, peak 1), catechin (tR = 16.03 min,
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Fig. 2. Total antioxidant activity of sugarcane measured by phosphomolybdenum reduction assay. (a) Antioxidant activity of different genotypes of sugarcane leaves (b) Antioxidant activity of different genotypes of sugarcane juice. Values are mean ± SD (n = 3).

peak 2), chlorogenic acid (t_R = 19.56 min, peak 3), caffeic acid (t_R = 22.17 min, peak 4), ferulic acid (t_R = 24.13 min, peak 5), epicatechin (t_R = 28.04 min, peak 6), ellagic acid (t_R = 32.17 min, peak 7), cuminum acid (t_R = 36.09 min, peak 8), rutin (t_R = 38.11 min, peak 9), quercitrin (t_R = 41.05 min, peak 10), isoquercitrin (t_R = 43.39 min, peak 11) and quercetin (t_R = 47.83 min, peak 12). The content
of phenolics and flavonoids are shown in Table 2. Ferulic acid (14.63 ± 0.03 mg/g), cumaric acid (11.65 ± 0.03 mg/g), quercetin (10.96 ± 0.02 mg/g), caffeic acid (9.16 ± 0.01 mg/g) and ellagic acid (9.03 ± 0.02 mg/g) were detected as major compounds. Whereas, catechin (4.51 ± 0.01 mg/g), epicatechin (5.78 ± 0.01 mg/g), gallic acid (4.38 ± 0.02 mg/g), quercetin (5.81 ± 0.01), isoquercetin (3.98 ± 0.01 mg/g), gallic acid (4.38 ± 0.02 mg/g) and chlorogenic acid (3.27 ± 0.02 mg/g) relatively showed minor contribution. This is the first comprehensive phytochemical study of sugarcane leaves. Earlier studies have shown the presence of phenolics, luteolin 8-C-rhamnosylglycoside, diosmetin-8-glucoside, vitexin, orinventin, tricin-7-O-neohesperidoside and tricin derivatives in the juice of sugarcane (Fahiana et al., 2008). Lee, Chen, Yu, Wang, and Duh (2012) reported the presence of flavonoids, benzoic acid, chlorogenic acid, caffeic acid and vitexin in leaves of different genotypes of sugarcane. The observed antioxidant activity and protecting ability of sugarcane against DNA damage is due to the phenolic acids (ferulic acid, gallic acid, chlorogenic acid, caffeic acid and cumaric acid) and flavonoids (quercetin, catechin, epicatechin, rutin and quercetin). The exogenous antioxidants from sugarcane extracts may act directly or indirectly with the internal antioxidant system for synergetic effects to protect several diseases linked to free radicals such as heart diseases, neurodisorders and other stress related disorders.

References


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