Antioxidative properties of *Canna edulis* Ker-Gawl.

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*Canna edulis* Ker- Gawl, found in the sub-Himalayan region contains starchy rhizome having economic importance in terms of food and herbal medicine. Correlation of phytochemical characteristics and antioxidative properties have been studied in hot and cold extracts of *C. edulis* rhizome. Antioxidants, BHT, quercetin, gallic acid and ascorbic acid equivalents have been determined using spectrophotometer. Hydrogen peroxide scavenging ability and reducing power properties have been used to define *in vitro* radical scavenging activity of both extracts. Total phenol and flavonoid contents were highest in hot extract (42.71 mg GAE/g and 21.92 mg QE/g) than cold extract (33.7 mg GAE/g and 15.12 mg QE/g). IC₅₀ value of DPPH and H₂O₂ were also observed to be higher in hot extract. The electron donation ability (EDA) of the hot extract (500 µg/ml) demonstrated stronger activity than cold extract (400 µg/ml). Further evaluation of free radicals was done by determining the specific Rf value of bioactive components through TLC. Thus the hot rhizome extract of *C. edulis* exerts more effective antioxidant property as compared to cold extract.

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**Introduction**

*Canna edulis* Ker-Gawl. is an edible perennial plant known for its rich starchy rhizome in the North eastern Himalayan regions of India¹. The plant has flat, broad, blackish-green leaves with small red flowers. Plant parts like rhizomes, leaves, flowers and seeds having considerable medicinal properties are used in various women health related issues and are also believed to be diuretic, demulcent and diaphoretic². Among various plant parts, the extracts of rhizomes are rich source of antioxidants which in turn provide evidences of biological activities of plants.

Oxidation is a necessary evil, which is essential for biological energy production in most of the living organisms, but excessive reactive oxygen species produced in various oxidative reactions are the main cause of some of the most critical diseases³. Antioxidants can terminate oxidative chain reactions by way of removing free radical intermediates by being oxidized themselves. Deficiency or misbalance of these networks in the human body has been found to be linked with diseases like cancer, atherosclerosis, diabetes, pre-mature ageing, etc. Many plants have been considered to be the major source of antioxidant production⁴⁵. The phenolic compounds and flavonoids present in these plants are known to be natural antioxidants having anticarcinogenic and anti-inflammatory properties⁶.

Though phenol and flavonoid contents of *C. indica* have been reported⁷⁸, no reports on presence of antioxidants in this plant found in sub Himalayan West Bengal and Darjeeling hills have been documented to date. In the present study, correlation of phytochemical characteristics and antioxidative properties of *C. edulis* has been taken up. Different parameters like presence of total phenols, flavonoids, ferrous reducing power, hydrogen peroxide scavenging assay of the rhizome of this plant present in the aforesaid regions have been investigated. Detection of antioxidant activities through DPPH scavenging assay and thin layer chromatography (TLC) has also been performed to study the different characteristics of these compounds.

**Materials and Methods**

**Plant material**

*C. edulis* was collected from hilly regions of Darjeeling, India in the autumn of 2008. The
authentication of the plant material was done at the Taxonomy and Environmental Biology Laboratory, University of North Bengal. The material has been deposited in the “NBU Herbarium” (accession no. 9588).

**Chemicals**

DPPH (2,2-diphenyl-1-picryl hydrazyl), gallic acid, ferric chloride, sodium nitrite were obtained from HiMedia Laboratories Pvt. Ltd, India, whereas methanol, KH₂PO₄, K₂HPO₄, NaOH, K₃Fe(CN)₆, ascorbic acid, trichloroacetic acid (TCA), quercetin, hydrogen peroxide, folin-Ciocalteu reagent, sodium carbonate (Na₂CO₃), butylated hydroxytoluene (BHT), aluminium chloride (AlCl₃), NH₃·H₂O, ethanol, ethyl acetate, formic acid, glacial acetic acid, etc. all were procured from Merck, India. The chemicals and solvents were of analytical grade.

**Rhizome extracts**

Twenty grams of rhizome of *C. edulis* was washed properly, dried and was crushed in 40 ml of double distilled water using mechanical grinder and finally 40 ml of double distilled water was added to get the required concentration of 1:4 (rhizome:H₂O).

**Cold extract (CeC)**

Half of the extract (20 ml) thus prepared was kept overnight in refrigerator for cold percolation. Next day, the sample was filtered using Whatman-2 filter paper. The filtrate was subjected to lyophilisation (Eyela Freeze Dryer FDU-506) to form powdery mass. Then required amount of double distilled water was added to those powdery extract to make a concentration of the stock solution to 1 mg/ml and stored at -20°C for further use.

**Hot extract (CeH)**

The remaining extract was subjected to exhaustive distillation with Soxhlet apparatus at boiling temperature for 3 hours. The extract was then lyophilized as described previously.

**DPPH radical scavenging activity:**

The free radical scavenging capacity of both the cold and hot extracts was determined using 2,2-diphenyl-1-picryl hydrazyl (DPPH)². DPPH solution (0.006% w/v) was prepared in 95% methanol. Freshly prepared DPPH solution was taken in test tubes and aqueous extracts of *Canna* rhizome was added followed by serial dilutions (100 µg to 1000 µg) in each test tube, to make a final volume of 2 ml. Discoloration of these extracts was measured at 517 nm (with Thermo UV1 spectrophotometer) after incubation for 30 min in dark at 37°C. Butylated hydroxytoluene (BHT) was used as a reference standard and dissolved in double distilled water to make the stock solution with the same concentration (1 mg/ml). Control sample was prepared containing the same volume without any extract. Methanol was used as blank. DPPH scavenging activity (%) was measured using the following equation:

\[ \text{DPPH scavenging activity } (\%) = \frac{A_0 - A_1}{A_0} \times 100 \]

where \( A_0 \) was the absorbance of the control and \( A_1 \) was the absorbance in the presence of the sample (aqueous rhizome extract of *Canna*). The actual decrease in absorption induced by the test compounds was compared with the positive controls. IC₅₀ value was calculated using the dose inhibition curve.

**Determination of total phenolic content**

Total phenolic content of both cold and hot extract were determined using Folin-Ciocalteu (FC) reagent method¹¹ with slight modification. The rhizome extracts (0.5 ml) was mixed with 0.5 ml of FC reagent (previously diluted 1:1 with double distilled water) and incubated for 5 min at room temperature (RT). Finally 1 ml of 20% Na₂CO₃ solution was added and incubated at RT for 10 mins. The absorbance was measured at 730 nm. Gallic acid monohydrate was used as standard. The total phenolic content was expressed as gram of gallic acid equivalents (GAE) per 100 gm extract.

**Determination of total flavonoid content**

The total flavonoid content was determined with aluminium chloride (AlCl₃) method¹² using quercetin as a standard. Both the cold and hot rhizome extract (0.25 ml each) were mixed with 1.25 ml double distilled water which was followed by the addition of 75µl of 5% NaNO₂. This mixture was incubated for 5 min at RT and then 0.15 ml of 10% AlCl₃ was added. The reaction mixture was treated with 0.5 ml of 1 mM NaOH after a incubation of 6 min at RT. Finally, the reaction mixture was diluted with 275 µl of double distilled water followed by an incubation of 20 min at RT. The absorbance was measured at 510 nm. The flavonoid content was calculated from a quercetin standard curve.

**Reducing power**

The reducing power of *C. edulis* rhizome extract was determined¹³. Different concentrations of extract
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(250 µg–2,500 µg), 1 ml each was taken in different tubes to which 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide \([K_3Fe(CN)_6]\) were added and incubated at 50°C for 20 min. 2.5 ml of TCA (10%) was added to the mixture. Since no precipitation occurred, 2.5 ml of the solution was mixed with double distilled water (2.5 ml) and FeCl\(_3\) (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as a reference standard. Phosphate buffer (pH 6.6) was used as blank solution. The absorbance of the final reaction mixture of two parallel experiments were taken and expressed as mean ± standard deviation.

**Hydrogen peroxide scavenging**

Hydrogen peroxide scavenging activity of *Canna* extract was determined\(^{14}\). Various concentrations (100-1000 µg/ml) of both cold and hot rhizome samples were mixed with 2 mM solution of H\(_2\)O\(_2\) in the ratio of 1:0.6 v/v and incubated for 10 min at RT. After incubation, absorbance of hydrogen peroxide at 230 nm was determined against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction. The percentage scavenging activity of hydrogen peroxide by *Canna* rhizome extract was calculated as follows:

\[
H_2O_2 \text{ scavenging activity (\%)} = \frac{Abs(\text{control}) - Abs(\text{standard})}{Abs(\text{control})} \times 100
\]

where, Abs (control): Absorbance of the H\(_2\)O\(_2\) (2 mM) as control and Abs (standard): Absorbance of the extract/standard.

**Electron donation ability (EDA) assay**

The EDA of sample was determined by the method of Kim et al\(^{15}\). This assay was based on the capacity of a substance to scavenge stable DPPH free radicals. The reaction mixture contained 1 ml of 0.15 mM DPPH-methanol solution, 3.98 ml of methanol, and 20 µl of different concentration samples and BHT (control). The mixture was allowed to react for 30 min at room temperature and the absorbance were measured at 517 nm using a spectrophotometer (Themo UV1 spectrophotometer). The EDA was expressed as the reduction rate of absorbance in accordance with the following equation:

\[
EDA(\%) = 1 - \frac{Abs(\text{sample})}{Abs(\text{control})} \times 100
\]

where, Abs (control): Absorbance of BHT as control and Abs (sample): Absorbance of the extract.

**Thin Layer Chromatography**

About 5 µl of the extract of both the cold and hot samples (100 mg/ml) were plotted on TLC plates (Merck, India 10×10 cm\(^2\)) using HPTLC plotter (Camag, Linomat-5). The plate was air dried and developed in suitable solvents for antioxidant compound detection containing ethyl acetate:glacial acetic acid:water in the ratio of 100:11:11:26, respectively. The plate was run in the above solvent for 30 min and dried at room temperature. Derivatization of the TLC plate was done by spraying DPPH solution at the concentration of 0.2% in methanol. The plate was dried in the hot oven at 100°C for 10 min. The bands were viewed under UV light at 365 nm. Different bands were observed and their corresponding Rf values were determined. All tests were performed in triplets.

**Statistical Analysis**

Results are expressed as mean ± S.E.M. of triplets. The groups were compared by two-way ANOVA using Graph Pad Prism, Version 5.0 (Graph Pad Software, San Diego, CA, USA). P-values < 0.001 were considered significant.

**Results and Discussion**

**Rhizome extract yield**

Rhizome extract yield was found to be 3.25 and 3.37% of cold and hot extract, respectively.

**Determination of total phenolic and flavonoids content**

The hot extracts have more phenolics and flavonoids as compared to cold extracts. The total phenolic content in the cold and hot extract of rhizome was 33.74 and 42.71 mg/ml gallic acid equivalent (GAE) per 100 mg of plant extract, respectively, whereas the flavonoids content were found to be 15.12 and 21.92 mg/ml quercetin equivalent per 100 mg plant extract. The increased in total phenol in hot extract may be due to release of low molecular weight phenolic compounds during heat treatment\(^{16}\).

**DPPH and EDA scavenging activity**

The *Canna* rhizome extracts were tested for their antioxidant properties in a range to determine their
potency to scavenge reactive oxygen species (ROS). The extracts exhibited a concentration dependent antiradical activity by quenching DPPH radical (Figure 1a) and EDA (Figure 1b). Here also we found that the hot extract exhibit higher antioxidant and electron donation ability than the cold extract. The high level of polyphenolic constituents in Canna can be attributed for its increased antioxidant activity during the DPPH assay. Specifically, hot rhizome extract having large amount of polyphenols is responsible for higher antioxidant activities and EDA in Canna. Both the above studied results were highly consistent with each other.

**Reducing power assay and hydrogen peroxide inhibitory activity**

The reducing power of Canna extract was determined from distinct colour changes (i.e. from yellow to green and blue) at 700 nm, depending on the reducing power of the sample concentration. The high absorbance of the reaction mixture indicates high reducing power. Figure 2a shows the dose-dependent reducing power of Canna hot and cold extract, which along with the positive control, increased steadily with increasing sample concentration. Hot extract (OD<sub>700</sub>=0.129) had higher levels of activity than cold extract (0.121 at OD<sub>700</sub>). It has also been observed that hot extract had higher hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) scavenging activity (Figure 2b) than that of the cold extract. Thus the above two results show the identical tendency as previously found in DPPH and EDA.

**IC<sub>50</sub> values in EDA, DPPH and Hydrogen peroxide scavenging activity**

Scavenging activity of aqueous extract of Canna rhizome was compared with standard BHT. The IC<sub>50</sub> values of cold and hot extract of Canna in EDA was 400 µg/ml and 500 µg/ml, respectively. In case of DPPH, IC<sub>50</sub> was found to be 570 µg/ml, 600 µg/ml, where as for H<sub>2</sub>O<sub>2</sub> scavenging test the values were

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**Figure 1**—(a) DPPH scavenging activity and (b) Electron donation ability (EDA) of Canna rhizome extract. CeC, Cold fraction; CeH, Hot fraction; BHT, butylated hydroxytoluene.

**Figure 2**— (a) Reducing power assay and (b) hydrogen peroxide scavenging activity of Canna rhizome extract. CeC, Cold fraction; CeH, Hot fraction; BHT, butylated hydroxytoluene.
1600 µg/ml, 1500 µg/ml, respectively for cold and hot extract. For standard BHT, these values were 700 µg/ml, 850 µg/ml and 1200 µg/ml, respectively for EDA, DPPH and hydrogen peroxide scavenging activity. The result is more or less as par with other Canna species.\(^7,9\)

**Linear correlation between hot and cold extracts of Canna**

In present study we found a close linear correlation between hot and cold extract of Canna rhizome. Strong correlation was observed in total phenol, DPPH and \(H_2O_2\) scavenging activity whereas it was found to be moderate in case of total flavonoids of both extracts. Figure 3 shows the significant correlation between hot and cold extracts for DPPH \((R^2=0.963)\), FRP \((R^2=0.872)\), \(H_2O_2\) \((R^2=0.998)\), TP \((R^2=0.978)\), TF \((R^2=0.614)\).

![Figure 3—Linear correlation coefficient of phenols, flavonoids and antioxidant activities of cold and hot of Canna rhizome extract.](image)

**Linear correlation between different parameters of hot and cold extract of Canna**

From these results it was seen that all the parameters were consistently highly correlated with each other in hot extract as compared to cold extract (Figure 4). It was found that total phenol was significantly positively correlated \([R^2(C)=0.552, R^2(H)=0.981]\) with DPPH in cold and hot extract, respectively. In case of \(H_2O_2\) scavenging activity and total phenol, a better linear relationship in hot extract as compared to cold extract was observed. The reducing power of both the extract were also positively correlated with hydrogen peroxide scavenging \([R^2(C)=0.973, R^2(H)=0.918]\) and total phenols \([R^2(C)=0.428, R^2(H)=0.974]\). For both cold and hot extracts the correlation of total flavonoids with DPPH was \([R^2(C)=0.510, R^2(H)=0.930]\) respectively and with reducing power was \((R^2(C)=0.337, R^2(H)=0.900)\) respectively. The close linkage between different parameters discussed above is due to the presence of high level of polyphenols in Canna rhizomes. The antioxidant activities of phenolic compounds are primarily due to their redox properties, which allow them to function as reducing agents, hydrogen donors and singlet-oxygen quenchers.\(^15\)

These observations clearly suggest a close linkage between total phenolic content and antioxidant activity, such as reducing power and scavenging effect on DPPH radicals.\(^18\)

**Thin layer chromatography**

Hot and cold extracts were subjected to thin layer chromatography (TLC) for rapid screening (Figure 5).

![Figure 4—Linear correlation coefficient in between phenols, flavonoids and antioxidant activities of cold and hot of Canna rhizome extract.](image)
The Rf values of the yellow spots with purple background on the TLC plate developed after the application of DPPH (0.2% in methanol) is the recognition of antioxidant molecules present in that extract.

**Conclusion**

The *in vitro* assay of Canna extracts illustrates the presence of high levels of polyphenols, flavonoids and antioxidants. In our study, a positive correlation was observed between phenols and flavonoids of Canna extracts with DPPH scavenging activity. A significant linear correlation was investigated between ferrous reducing power and hydrogen peroxide inhibitory effects when compared with phenols and flavonoids. TLC chromatograms showed the presence of different compounds which might have potential value for drugs intelligence activities. This report also indicates that hot extract of Canna gives more effective antioxidant property as compared to cold percolated extract. Oxidative stress can also be effectively attenuated via its antioxidant property. However, further studies are needed to isolate active principle responsible for the overall activity of both the extracts.

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