

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.

Keywords: *Canna edulis*, Antioxidants, Flavonoids, Phenols, Edible rhizome.

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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 production^{4,5}. 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 reported⁷⁻⁹, 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

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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, KH_2PO_4 , K_2HPO_4 , NaOH, $\text{K}_3\text{Fe}(\text{CN})_6$, ascorbic acid, trichloroacetic acid (TCA), quercetin, hydrogen peroxide, folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), butylated hydroxytoluene (BHT), aluminium chloride (AlCl_3), NH_4OH , 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: H_2O).

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)¹⁰. 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 Themo 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. IC_{50} 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% Na_2CO_3 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 (AlCl_3) 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 75 μl of 5% NaNO_2 . This mixture was incubated for 5 min at RT and then 0.15 ml of 10% AlCl_3 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

(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 [K₃Fe(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 FeCl₃ (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 determined¹⁴. Various concentrations (100-1000 µg/ml) of both cold and hot rhizome samples were mixed with 2 mM solution of H₂O₂ 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 scavenging activity (%) =

$$\frac{Abs(control) - Abs(standard)}{Abs(control)} \times 100$$

where, Abs (control): Absorbance of the H₂O₂ (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:

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

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 cm²) using HPTLC plotter (Camag, Linomat-5). The plate was air dried and developed in suitable solvents for antioxidant compound detection containing ethyl acetate:formic acid: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 R_f 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 treatment¹⁶.

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 activities¹⁷ 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 (OD₇₀₀=0.129) had higher levels of activity than cold extract (0.121 at OD₇₀₀). It has also been observed that hot extract had higher hydrogen peroxide (H₂O₂) 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.

IC₅₀ values in EDA, DPPH and Hydrogen peroxide scavenging activity

Scavenging activity of aqueous extract of *Canna* rhizome was compared with standard BHT. The IC₅₀ values of cold and hot extract of *Canna* in EDA was 400 µg/ml and 500 µg/ml, respectively. In case of DPPH, IC₅₀ was found to be 570 µg/ml, 600 µg/ml, where as for H₂O₂ scavenging test the values were

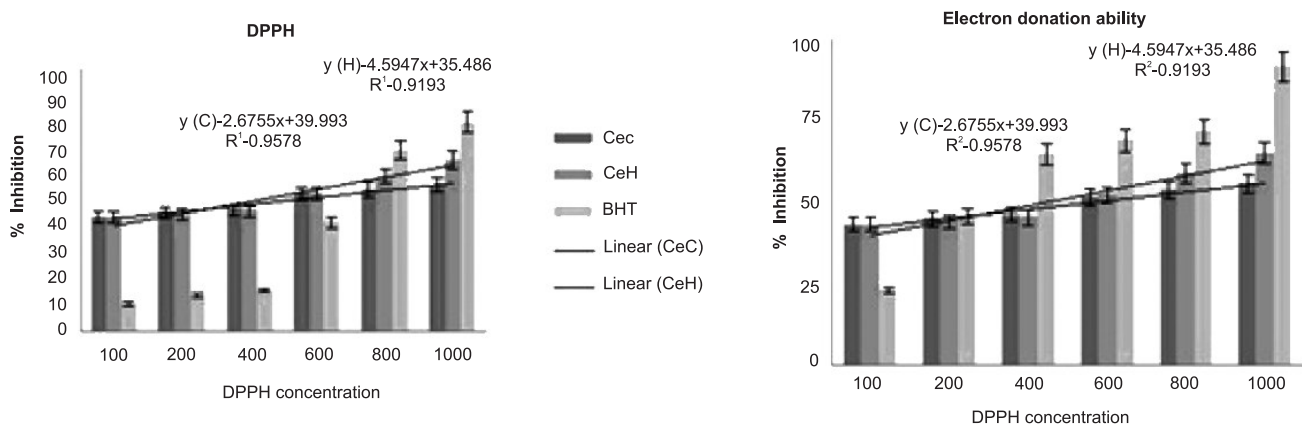


Figure 1—(a) DPPH scavenging activity and (b) Electron donation ability (EDA) of *Canna* rhizome extract. CeC, Cold fraction; CeH, Hot fraction; BHT, butylated hydroxytoluene.

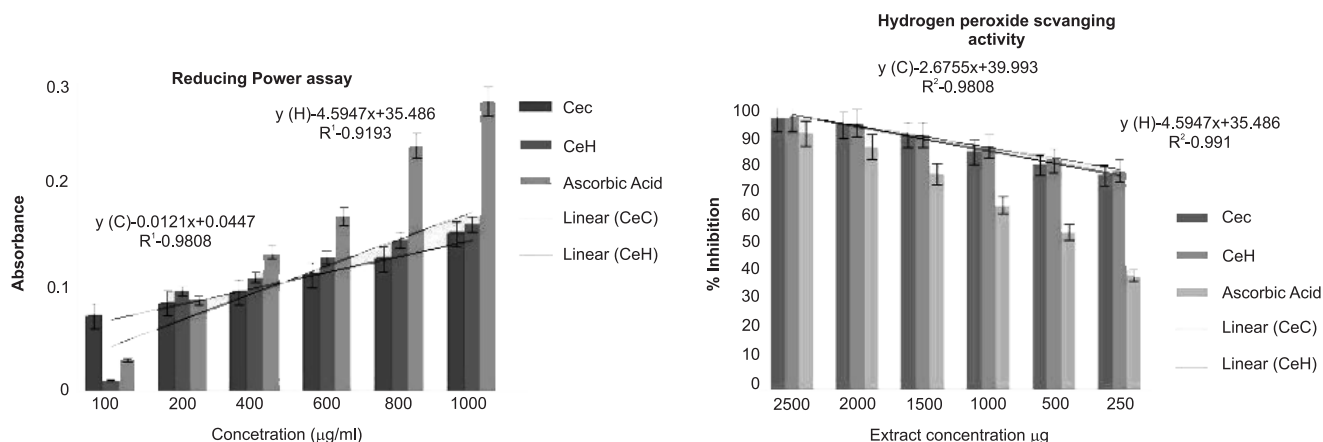


Figure 2—(a) Reducing power assay and (b) hydrogen peroxide scavenging activity of *Canna* rhizome extract. CeC, Cold fraction; CeH, Hot fraction; BHT, butylated hydroxytoluene.

1600 $\mu\text{g/ml}$, 1500 $\mu\text{g/ml}$, respectively for cold and hot extract. For standard BHT, these values were 700 $\mu\text{g/ml}$, 850 $\mu\text{g/ml}$ and 1200 $\mu\text{g/ml}$, 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 ($R^2=0.614$).

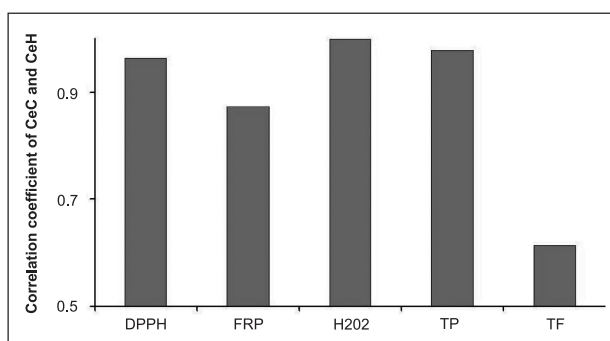


Figure 3—Linear correlation coefficient of phenols, flavonoids and antioxidant activities of cold and hot of *Canna* rhizome extract.

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 [$R^2(\text{C})=0.552$, $R^2(\text{H})=0.981$] 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 [$R^2(\text{C}) 0.973$, $R^2(\text{H})=0.918$] and total phenols [$R^2(\text{C})=0.428$, $R^2(\text{H})=0.974$]. For both cold and hot extracts the correlation of total flavonoids with DPPH was [$R^2(\text{C})=0.510$, $R^2(\text{H}) =0.930$] respectively and with reducing power was ($R^2(\text{C})=0.337$, $R^2(\text{H})0.900$) 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 quenchers¹⁵. These observations clearly suggest a close linkage between total phenolic content and antioxidant activity, such as reducing power and scavenging effect on DPPH radicals¹⁸.

Thin layer chromatography

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

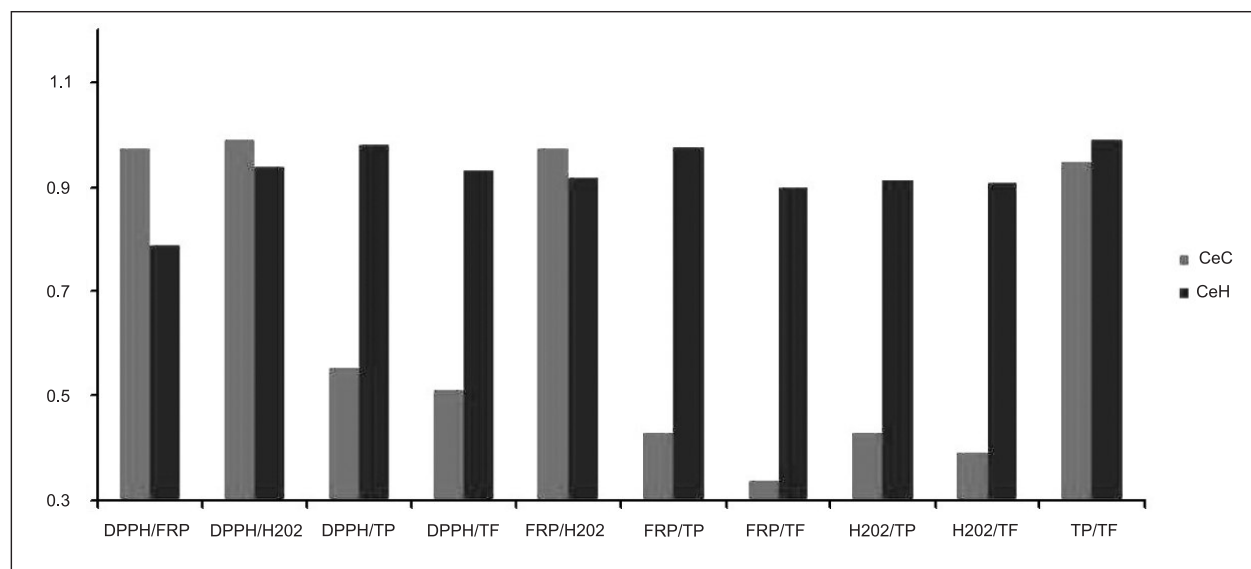


Figure 4—Linear correlation coefficient in between phenols, flavonoids and antioxidant activities of cold and hot of *Canna* rhizome extract.

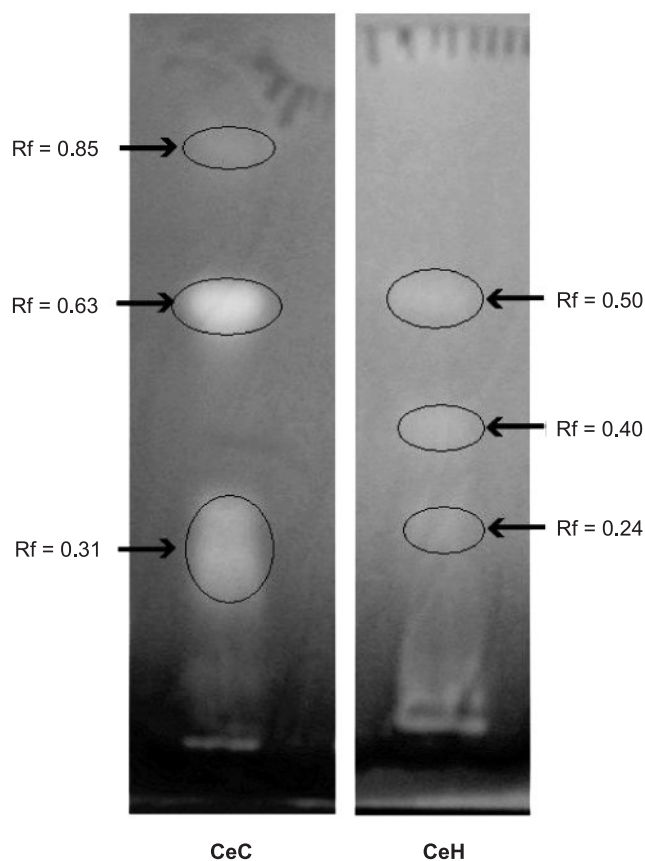


Figure 5—Rapid screening by TLC.

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 extract¹⁹.

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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References

- 1 Tanaka N, The utilization of edible *Canna* plants in southeastern Asia and southern China, *Econ Bot*, 2004, **58**(1), 112-114.
- 2 Duke J A and Ayensu E S, Medicinal plants of China, Vol 1, Reference Publications (Algonac, Michigan), 1985, 232.
- 3 Oboh G, Adefegha S A, Ademosun A O and Unu D, Effects of hot water treatment on the phenolic phytochemicals and antioxidant activities of lemon grass (*Cymbopogon citratus*), *Electr J Envir Agric Food Chem*, 2010, **9**(3), 503-513.
- 4 Larson R A, The antioxidants of higher plants, *Phytochemistry*, 1988, **27**, 969-973.
- 5 Sushil Kumar Middha1, Yashoda Mittal, Talembedu Usha, Dharmendra Kumar, Ranjani Srinivasan, Laxmikant Vashisth1, Biplab Bhattachargae and Madhugiri Bhojanganarao Nagaveni, *Phyto-mellitus: A phyto-chemical database for diabetes*, *Bioinformation*, 2009, **4**(2), 78-79.
- 6 Mukhtar H, Katiyar S K and Agarwal R, Green tea and skin-anticarcinogenic effect, *J Invest Dermatol*, 1994, **102**(1), 3-7.
- 7 Atrooz O M, The incorporation effects of methanolic extracts of some plant seeds on the stability of phosphatidylcholine liposomes, *Pak J Biol Sci*, 2007, **10**(10), 1643-1648.
- 8 Vankar P S and Srivastava J, Comparative study of total phenol, flavonoid contents and antioxidant activity in *Canna indica* and *Hibiscus rosa-sinensis*: Prospective natural food dyes, *Int J Food Eng*, 2008, **4**(3), DOI: 10.2202/2008.1556-3758.1232.
- 9 Joshi Y M, Kadam V J and Kaldhone P R, *In vitro* antioxidant activity of methanolic extract of aerial parts of *Canna indica* L., *J Pharm Res*, 2009, **2**(11), 1712-1715.
- 10 Hasan MS, Ahmed M I, Mondal S, Uddin S J, Masud M M, S K Sadhu and M Ishibashi, Antioxidant, antinociceptive activity and general toxicity study of *Dendrophthoe falcata* and isolation of quercitrin as the major component, *Orient Pharm Exp Med*, 2006, **6**(4), 355-360.
- 11 Singleton VL and Rossi JA, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, *Am J Enol Viticult*, 1965, **16**(3), 144-158.
- 12 Goyal AK, Middha SK and Sen A, Evaluation of the DPPH radical scavenging activity, total phenols and antioxidant activities in Indian wild *Bambusa vulgaris* "Vittata" methanolic leaf extract, *J Nat Pharm*, 2010, **1**(1), 40-45.
- 13 Oyaizu M, Studies on products of browning reaction prepared from glucoseamine, *Jpn J Nutr*, 1986, **44**, 307-314.
- 14 Ruch RJ, Cheng S J and Klaunig J E, Prevention of cytotoxicity and inhibition of intracellular communication by

- antioxidant catechins isolated from Chinese green tea, *Carcinogenesis*, 1989, **10**(6), 1003-1008.
- 15 Ju-Sung Kim, Yong-Soo Kwon, Wan-Joo Chun, Tae-Young Kim, Jianhua Sun, Chang-Yeon Yu and Myong-Jo Kim, *Rhus verniciflua* Stokes flavonoid extracts have antioxidant, antimicrobial and α -glucosidase inhibitory effect, *Food Chem*, 2010, **120**, 539-543.
 - 16 Xu G, Ye X, Chen J and Liu D, Effect of heat treatment on the phenolic compounds and antioxidant capacity of citrus peel extract, *J Agric Food Chem*, 2007, **55**, 330-335.
 - 17 Rice-Evens C A, Miller N J and Paganga G, Antioxidant properties of phenolic compound, *Trends Plant Sci*, 1997, **2**(4), 152-159.
 - 18 Do J R, Kim K J, Park S Y, Lee O H, Kim B S and Kang S N, Antimicrobial and antioxidant activities of ethanol extracts of medicinal plants, *J Food Sci Nutr*, 2005, **10**(1), 81-87.
 - 19 Conforti F, Marrelli M, Statti G and Menichini F, Antioxidant and cytotoxic activities of methanolic extract and fractions from *Senecio gibbosus* subsp *gibbosus* (Guss) DC., *Nat Prod Res*, 2006, **20**(9), 805-812.