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Antioxidant Activity and Trace Elements Profile of Extracts of *Cymbopogon jwarancusa* (Jones.) leaves

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ABSTRACT

This research paper report the antioxidant activity and trace element analysis of *Cymbopogon jwarancusa* (Jones.). The ethanol extract showed highest antioxidant activity in DPPH (32.08 ± 0.53 % inhibition) and FRAP (39.01±0.64 Fe (II) micromole per litre) assay while water extract showed highest antioxidant activity in βCL (54.26±1.09 %) at 1mg/10ml concentration. BHT and ascorbic acid were used as standards. Trace elements analysis showed sodium (Na^{+}) 0.08%, potassium (K^{+}) 0.05%, lithium (Li^{+}) below detection limit, , nickel (Ni^{++}) 0.03%, lead (Pb^{++}) 0.04%, cadmium (Cd^{++}) 0.08%, zinc (Zn^{++}) 0.08%, copper (Cu^{++}) 0.05%, manganese (Mn^{++}) 0.18%, iron (Fe^{++}) 0.22%, and Cobalt (Co^{++})0.08%, Calcium (Ca^{++}) 0.50%, Magnesium (Mg^{++})0.02%, Silicon (Si^{+4}) 0.24%, Phosphorus (P^{-3}) 0.08% and Sulphur (S^{--})0.01%. were determined.

Keywords: *Cymbopogon jwarancusa*, Antioxidant, Trace Elements, DPPH, FRAP, βCL.

1. Introduction

Plants play as important role in our life. Plants not only provide us nutrition but also they have medicinal values. Herbs are being used by about 80% of the world population especially in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and minimal side effects [25]. *Cymbopogon jwarancusa* (Jones.) is an aromatic grass belonging to the *Poaceae* family. The species name has been derived from two Sanskrit words "Jwar & Ankusha" meaning "fever and breaker" respectively that recalls the much acclaimed medicinal property associated with the herb. In the recent years, several researchers have reported the usage of this plant against different diseases like vomiting, abdominal tumors, unconsciousness, blood impurities, skin problems etc [26]. The medicinal properties are attributed to be the outcome of biochemical composition of the species. No work has been reported on the antioxidant and Trace metals profiles of this grass. Hence an attempt has been made to trace out the antioxidant properties and trace elements of water and ethanol extracts of the aromatic leaves from the plant.

2. Materials and Methods

2.1 Plant Material and Extraction Procedures

Cymbopogon jwarancusa (Jones.) leaves were collected from cantonment area of Kanpur, Uttar Pradesh, India. Whole plant was washed and dried in shade. The dried sample was than ground, powder sample was used for analysis. An initial quality evaluation of the plant material was carried out as per the guidelines on herbal quality control [37] and a voucher specimen (C1/Chem/DAV/11) has been deposited in the Department of Chemistry, Dayanand Anglo Vedic (DAV) College, Kanpur, Uttar Pradesh, India for further reference. Oil was extracted from leaf by using a soxhlet apparatus. Trace elements were determined on Inductive Coupled Plasma – Optical Emission Spectrometer (ICP-OES Perkin Elmer Optima 5300-V, USA). Antioxidant is determined by UV-Vis Spectrophotometer (Shimadzu UV-1800, Japan). Extracts were prepared according to Oke & Mhamburger [31].

2.2 Chemicals

The chemicals used were 2,2-diphenyl- 1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), β-carotene (Sigma Aldrich, USA), ascorbic acid (Merck Darmstadt, Germany)**,** tris HCl, sodium acetate trihydrated, glacial acetic acid, ferric chloride hexahydrated ($FeCl_3.6H_2O$), ferrous sulphate heptahydrated ($FeSO_4.7H_2O$), ethanol and linoleic acid, tween 20, butylated hydroxyl toluene (BHT), and other chemicals used were of analytical grade and were obtained from either Sigma-Aldrich or Merck.

2.3 DPPH Free Radical Scavenging Assay

The free radical scavenging capacity of water and ethanol extract of *C. jwarancusa* leaves was evaluated with the methodology described by Blois **[1958]** as elaborated by Elmastas **[18]**. The solution of DPPH (0.1 mM) was prepared and 3 ml of DPPH solution was added to 0.1 ml of the solution of water or ethanol at different concentrations (*viz*. 0.25, 0.5, 1.0, and 2.0 mg/10 ml). The absorption was measured at 515 nm up to 30 min or until it remained constant. The scavenging capacity of DPPH radical was calculated using the following formula: **[20]**.

$$\text{Inhibition of DPPH (\%)} = \left(\frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \right) x100$$

Where, $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of water or ethanol extract. BHT and ascorbic acid were used as standards.

2.4 Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP value was calculated using the formula described by Benzie and Strain (1996), which was based on reduction of Fe^{+3} TPTZ to a blue coloured Fe^{+2} TPTZ. The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ and 20 mM $FeCl_3.6H_2O$ in a ratio 10:1:1 at 37 ºC. The absorbance readings were taken after 0.5 s and every 15s until 4min and the absorbance was measured at 593 nm. The change of absorbance ΔA = A4min - A0min was calculated and compared to ΔA of Fe^{+2} standard solution. The antioxidant potential of samples was determined from a standard curve and plotted using $FeSO_4.7H_2O$ at a concentration range between 200 and 1000 μM.

2.5 β - Carotene Linoleate Bleaching (βCL) Assay

Total antioxidant activity of *C. jwarancusa* extracts and standards (BHT & ascorbic acid) was measured by standard methods **[24]**. One mililitre of β-carotene solution (0.2 mg/ml chloroform) was pipetted into a round bottom flask (100 ml) containing 0.02 ml of linoleic acid and 0.2 ml of 100 % Tween-20. The mixture was then evaporated at 40 ºC for 10 min by rotary evaporator to remove chloroform. Then the mixture was immediately diluted with 100 ml of distilled water. The distilled water was added slowly to the mixture with vigorous agitation to form an emulsion. Five ml of emulsion was transferred into test tube containing 0.2 ml of samples in 80% methanol at different tested concentrations. The tubes were then gently agitated and incubated at 45 ºC for 2 hrs. The absorbance of the samples was measured at 470 nm using a spectrophotometer at initial time (t = 0) against a blank, consisting of an emulsion without β-carotene. The standards at the same concentration with samples were used as comparison. 0.2 ml of 80% methanol in 5 ml of the above emulsion was used as control. The observations were carried out at 15 min interval. Antioxidant activity was measured in terms of successful bleaching of β-carotene in percentage using the formula:

$$AA = \left(1 - \frac{A_0 - A_t}{A_0^0 - A_t^0} \right) x100$$

Where, A_0 and A_{00} are the absorbance values measured at initial time of the incubation for samples and control respectively, while At and are the absorbance values measured in the samples or standards and control at t = 120 min.

2.6 Trace Elements Analysis

The trace elements analysis of *C. jwarancusa* leaves was evaluated with the methodology as elaborated by UOP Method 389 (2004). The sample is coked with fuming sulfuric acid, ignited and ashed at 538 ºC. The residue is then treated with aqua regia and, after evaporation, is dissolved in dilute hydrochloric acid containing scandium as internal standard. The concentrations of elements in the resulting solutions are determined by Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES). This method is for determining iron (Fe), nickel (Ni), vanadium (V), lead (Pb), copper (Cu), sodium (Na), molybdenum (Mo), manganese (Mn), chromium (Cr), magnesium (Mg), tin (Sn), calcium (Ca), aluminum (Al), and zinc (Zn) in oils. Most of the elements can be determined within the concentration range of 0.01 to 200 mass - ppm when a 50-g sample of oil is ashed. Higher or lower concentrations can be determined by ashing appropriate-sized samples and/or modifying the instrument operating parameters. Determination of additional elements is possible if they are not volatilized during the ashing step and do not form insoluble sulfates.

2.7 Statistical Analysis

The assays were carried out in triplicate and the results were expressed as means ± standard errors. The antioxidant activity and trace elements of the extracts were analyzed using analysis of variance (ANOVA). The statistical analyses were carried out using Origin 8 (Northampton, MA01060, USA).

3. Results and Discussion

3.1 DPPH Free Radical Scavenging Assay

The extracts found in increasing order of inhibition of DPPH were Water extract < Ethanol extract < BHT < Ascorbic acid (Table 1).

Table 1. Antioxidant activity of ethanol and water extracts of *Cymbopogon jwarancusa* (Jones.) leaves by DPPH assay in terms of percentage inhibition of DPPH (Mean ± SE_{mean}).

S. No.	Concentrations (mg/10ml)	Ethanol extract	Water extract	Ascorbic acid	BHT
1	0.25	10.98± 0.60	08.32± 0.09	12.69 ± 0.48	20.02 ± 1.26
2	0.50	23.53 ± 0.66	17.37± 0.57	24.53 ± 1.04	43.51 ± 1.12
3	1.00	32.08 ± 0.53	25.99 ± 1.10	43.01 ± 0.62	55.34 ± 1.42
4	2.00	45.06 ± 0.53	36.45 ± 0.30	93.10 ± 0.35	76.05 ± 0.76

ANOVA followed by Tukey test; values in rows followed by same letters are not significantly different (p <0.05).

The results showed that the % inhibition of DPPH free radicals were increased according to their concentrations. There was a significant difference (P<0.05) between the percent inhibition of DPPH of extracts and concentrations. The free radical scavenging activity of *C. jwarancusa* was significantly higher than the reported values for *Ocimum basilicum* [23], but was lower in *Morchella conica* with the free radical eradication [35]. The ethanol extracts of *C. jwarancusa* leaves showed highest antioxidant activity in this assay.

Hydrogen–donor capacities of polyphenols for DPPH radical were found proportional to the number of hydroxyl groups [27] and the amount of inactivated DPPH radical was found proportional to the concentration of added flavonoids. The observed lowest inhibition values of the extract may be due to the fact that DPPH· is a long lived less reactive radical, which reacts only with very reactive phenolic and other antioxidants.

3.2 Ferric Reducing Antioxidant Power (FRAP) assay

The ethanolic extract of *C. jwarancusa* exhibited higher antioxidant potential than water extract. The increasing order of reducing ability were found as Water extract < Ethanol extract < Ascorbic acid < BHT at 1mg/10ml (Table 2). $FeSO_4.7H_20$ was used for calibration (R2 = 0.98).The reducing properties are associated with the presence of compounds which exert their action by breaking the free radical chain through donating a hydrogen atom [21, 16]. This suggested the presence of inhibiting compounds as a result of FRAP activity in different extracts. The FRAP assay is widely used in the evaluation of antioxidant components in dietary polyphenols. The antioxidant activity increases proportionally to polyphenol contents and according to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in numerous plant species [1].

Table 2: Antioxidant activity of ethanol and water extracts of *Cymbopogon jwarancusa* (Jones.) leaves by FRAP assay in terms of Fe (II) micromole per litre (Mean ± SE_{mean}).

S. No.	Concentrations (mg/10ml)	Ethanol extract	Water extract	Ascorbic acid	BHT
1	0.25	22.32± 0.34	13.75± 0.43	21.92 ± 0.47	33.01 ± 0.92
2	0.50	30.06 ± 0.23	20.72± 0.69	34.07 ± 0.98	43.68 ± 0.72
3	1.00	39.01 ± 0.64	36.04 ± 0.65	46.97 ± 1.27	52.08 ± 0.77
4	2.00	47.71 ± 1.02	46.26 ± 0.71	85.06 ± 1.02	77.56 ± 1.13

ANOVA followed by Tukey test; values in rows followed by same letters are not significantly different (p <0.05).

3.3 β - Carotene Linoleate Bleaching (βCL) Assay

The antioxidant activities of the solvent extracts in βCL assay were observed in increasing order as Ethanol extract < Water extract < BHT < Ascorbic acid in all concentrations (Table 3). The ethanol extract was found to be the most effective. It is clear that the presence of antioxidants in the leaves of *C. jwarancusa* extracts reduced the oxidation of β-carotene. The control sample oxidized most rapidly. There were significant differences (p<0.05) between the different extracts and standards with concentrations. Amin I and Tan S.H [5] also found similar results in water and alcoholic extract while working with seaweeds like species of *Laminaria*, *Undaria* and *Hijiki*.

Table 3: Antioxidant activity of ethanol and water extracts of *Cymbopogon jwarancusa* (Jones.) leaves by βCL assay in terms of percent (Mean ± SE_{mean}).

S.No.	Concentrations (mg/10ml)	Ethanol extract	Water extract	Ascorbic acid	BHT
1	0.25	21.74± 0.63	21.35± 1.14	23.93 ± 0.33	18.68 ± 1.25
2	0.50	36.06 ± 1.02	38.82± 1.22	42.98 ± 0.20	40.72 ± 0.92
3	1.00	47.13 ± 1.19	54.26 ± 1.09	94.29 ± 0.28	88.89 ± 1.36
4	2.00	55.93 ± 0.78	65.12± 1.03	99.69 ± 0.39	96.58 ± 0.63

ANOVA followed by Tukey test; values in rows followed by same letters are not significantly different (p <0.05).

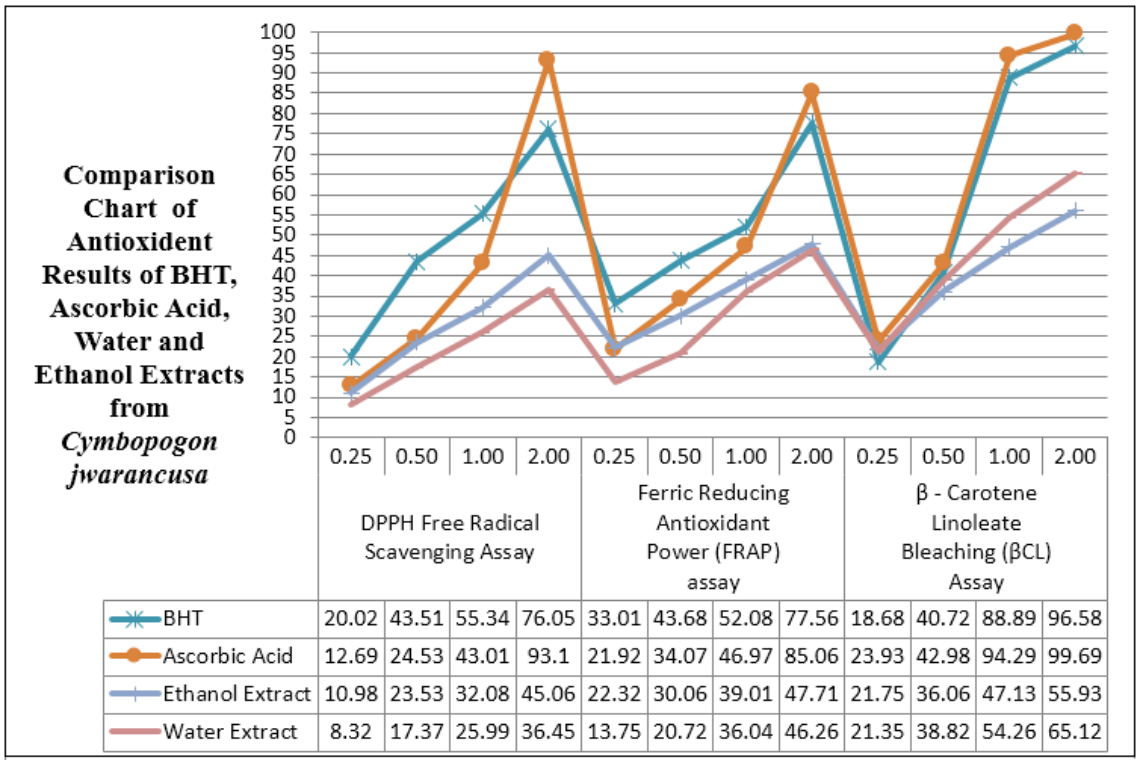
3.4 Trace Elements Analysis

Trace elements analysis are shown in Table 4, which indicated that sodium (Na^+) 0.08%, potassium (K^+) 0.05%, lithium (Li^+) below detection limit, , nickel (Ni^{++}) 0.03%, lead (Pb^{++}) 0.04%, cadmium (Cd^{++}) 0.08%, zinc (Zn^{++}) 0.08%, copper (Cu^{++}) 0.05%, manganese (Mn^{++}) 0.18%, iron (Fe^{++}) 0.22%, Cobalt (Co^{++})0.08%, Calcium (Ca^{++}) 0.50%, Magnesium (Mg^{++}) 0.02%, Silicon (Si^{+4}) 0.24%, Phosphorus (P^{-3})0.08% and Sulphur (S^{--})0.01%. Trace quantity of

this elements are essential for enzymatic processes of biological systems. The elements are made available to human body by the plant kingdom and hence their presence is vital for the health of body and for the cure diseases.

Table 4: Trace Elements Analysis of ethanol and water extracts of *Cymbopogon jwarancusa* (Jones.)

Elements	Result (Percentage)
Na	0.08
K	0.05
Li	0.01
Ni	0.03
Pb	0.04
Cd	0.08
Zn	0.08
Cu	0.05
Mn	0.18
Fe	0.22
Co	0.08
Ca	0.50
Mg	0.02
Si	0.24
P	0.08
S	0.01



Graph: Comparison Chart of Antioxidant Results of BHT, Ascorbic Acid, Water and Ethanol Extracts from Cymbopogon jwarancusa

4. Conclusions

The results obtained in this study clearly showed that water and ethanol extracts have powerful antioxidant activity against various antioxidant systems *in vitro.* Moreover, these extracts can be used as easily acceptable source of natural antioxidants and as a possible food supplement or may be useful in pharmaceutical applications. Further studies related to the identification and evaluation of natural antioxidant compounds from plant extracts would give further impetus to antioxidant therapy by providing new drug candidates. The result of trace elements indicated the importance of medicinal plant, which can be used in curing of different disease sometimes different combination of mineral element present in medicinal plant may be essential for the cure of diseases.

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