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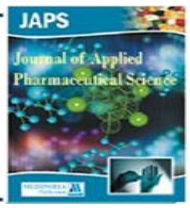

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Antioxidant and antimicrobial activity of ethanol and water extracts of *Cymbopogon jwarancusa* (Jones.) leaves

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ABSTRACT

The *Cymbopogon jwarancusa* (Jones.) leaves extracts were explored for their antioxidant and antimicrobial activity. The ethanol extract showed highest antioxidant activity in DPPH (31.99 ± 0.50 % inhibition) and FRAP (38.79 ± 0.54 Fe (II) micromole per litre) assay while water extract showed highest antioxidant activity in βCL (54.76 ± 1.37 %) at 1mg/10ml concentration. BHT and ascorbic acid were used as standards. During antimicrobial assay, the ethanol extract found was very effective against *Fusarium oxyporium* f.sp-*lini* (85.31 ± 0.25 mm) and *Staphylococcus aureus* (94.37 ± 0.28 mm) at 500 ppm while water extract was found less effective against *Aspergillus flavus* (03.72 ± 0.19 mm) at 100 ppm concentration. The ethanol extract can be used for the applications such as food preservation as a remedy and against dental diseases.

Keywords: *Cymbopogon jwarancusa*, Antioxidant, Antimicrobial, DPPH, FRAP, βCL.

INTRODUCTION

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 (Kamboj, 2000). *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 tumers, unconsciousness, blood impurities, skin problems etc. (Kirtikar and Basu, 1982). The medicinal properties are attributed to be the outcome of biochemical composition of the species. Mahmud *et al.,* (2002) have reported the trace element profile of *C. jwarancusa* but no work has been reported on the antioxidant and antimicrobial activities of this grass. Hence an attempt has been made to trace out the antioxidant and antimicrobial properties of water and ethanol extracts of the aromatic leaves from the plant.

MATERIALS AND METHODS

Plant Material and Extraction Procedures

Cymbopogon jwarancusa (Jones.) leaves were collected from cantonment area of Kanpur, Uttar Pradesh, India. An initial quality evaluation of the plant material was carried out as per the guidelines on herbal quality control (WHO,1998) and a voucher specimen (C1/Chem/DAV/11)

has been deposited in the Department of Chemistry, Dayanand Anglo Vaidic (DAV) College, Kanpur, Uttar Pradesh, India for further reference. The extracts were prepared according to Oke & Mhamburger (2002).

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.

Test Microorganisms and Growth Media

The microbiological samples *Staphylococcus aureus* and *Streptococcus salivarius* were collected from Microbiology division of Shivam Dental Hospital, Kanpur, Uttar Pradesh, India. The isolates were identified according to published guidelines of Burneti *et al.*, 1994. The fungal isolates *Fusarium oxysporum* f sp. Lini splini and *Aspergillus flavus*, were taken from the plant pathology laboratory, Dayanand Anglo Vaidic College, Kanpur, Uttar Pradesh, India. The bacterial and fungal strains were maintained on Mueller–Hinton agar (MHA) and Potato dextrose agar (PDA) plates respectively at 4 °C.

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 (2006). 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: (Gulcin *et al.*, 2005 c).

$$\% \text{ Inhibition of DPPH} = \left(\frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \right) \text{x } 100$$

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.

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^{0}C. The absorbance readings were taken after 0.5s and every 15s until 4min and the absorbance was measured at 593nm. The change of absorbance $\Delta A = A_{4min} - A_{0min}$ 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.

β - Carotene Linoleate Bleaching (βCL) Assay

Total antioxidant activity of *C. jwarancusa* extracts and standards (BHT & ascorbic acid) was measured by standard methods (Jayaprakasha *et al.*, 2001). 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 ^{0}C 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 ^{0}C 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:

$$\% \text{ AA} = \left(1 - \frac{A_0 - A_t}{A_0^0 - A_t^0} \right) \text{x} 100$$

where A_0 and A_0^0 are the absorbance values measured at initial time of the incubation for samples and control respectively, while A_t and A_t^0 are the absorbance values measured in the samples or standards and control at t = 120 min.

Antimicrobial Assay

The antimicrobial activity was evaluated by food poison technique which has been described by Nene and Thapliyal (1993). The water and ethanol extract were used to prepare 100, 200 and 500 ppm concentrations of nutrient agar for antibacterial and PDA for antifungal assay. The control plates were poured with the respective medium without plant material. The 16 hrs culture of *Staphylococcus aureus* and *Streptococcus salivarius* were diluted with sterile physiological saline solution (PS: 0.85% w/v sodium chloride) so that a concentration of inoculum approximately 10^8 cfu mL^{-1} could be achieved. The Whatman filter paper disc of 5 mm diameter was dipped in this bacterial growth and was placed in the centre of the above prepared nutrient agar plate. PDA petriplates containing different concentrations of plant material and were inoculated with a 5 mm mycelial disc of the fungal species from 7 day old culture grown on PDA. The inoculated plates were incubated at 25 ± 1^{0}C till the fungus covered the control plates. The colony diameter was recorded and percent inhibition in each

treatment was calculated. The zones < 8 mm were not considered significant.

Statistical Analysis

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

RESULTS AND DISCUSSION

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}).

Entry	Concentrations (mg/10ml)	Ethanol extract	Water extract	Ascorbic acid	BHT
01	0.25	11.11 ± 0.66[a]	8.20 ± 0.12	12.56 ± 0.28[a]	19.72 ± 1.46
02	0.50	23.39 ± 0.64[b]	17.25 ± 0.53	24.31 ± 1.34[b]	43.31 ± 1.01
03	1.00	31.99 ± 0.50	26.11 ± 1.20	42.81 ± 0.59	55.74 ± 1.73
04	2.00	44.83 ± 0.56	36.23 ± 0.20	93.40 ± 0.15	75.88 ± 0.86

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

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}).

Entry	Concentrations (mg/10ml)	Ethanol extract	Water extract	Ascorbic acid	BHT
01	0.25	22.26 ± 0.27[a]	13.45 ± 0.30	21.61 ± 0.39[a]	32.73 ± 0.83
02	0.50	29.98 ± 0.53	20.53 ± 0.82	33.98 ± 1.05	43.47 ± 0.51
03	1.00	38.79 ± 0.54	35.82 ± 0.75	47.02 ± 1.53	51.80 ± 0.61
04	2.00	47.43 ± 0.92[b]	46.42 ± 0.82[b]	84.78 ± 0.88	77.29 ± 1.24

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* (Gulecin *et al.*, 2007), but was lower in *Morchella carica* with the free radical eradication (Turkoglua *et al.*, 2006).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 (Lee and Shibamoto, 2001) 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.

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 (R^2 = 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 (Gordon, 1990; Duh *et al.*, 1999).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 (Adeolu *et al.*, 2009).

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 oxidised most rapidly. There were significant differences (p<0.05) between the different extracts and standards with concentrations. Amin I and Tan S.H (2002) 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}).

Entry	Concentrations (mg/10ml)	Ethanol extract	Water extract	Ascorbic acid	BHT
01	0.25	21.52 ± 0.98[a]	21.35 ± 1.03[a]	23.74 ± 0.25	18.54 ± 1.33
02	0.50	35.75 ± 0.96	38.45 ± 1.11	43.18 ± 0.11	40.57 ± 0.81
03	1.00	46.82 ± 1.25	54.76 ± 1.37	94.67 ± 0.14	88.46 ± 1.55
04	2.00	55.72 ± 0.86	64.92 ± 1.10	99.30 ± 0.28	96.43 ± 0.56

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

Antimicrobial Activity

The preliminary antimicrobial screening of *C. jwarancusa* leaves in water and ethanol extracts gave relatively wide inhibition zones against the test strains as compared to positive control (Table 4). The tested microbes belonging to the indigenous or resident oral microorganisms can led to infectious dental diseases. More than 25% of the world cereals have been found contaminated with known mycotoxins (especially aflatoxins) and more than 300

Table 4. Antimicrobial activity of water and ethanol extracts of *Cymbopogon jwarancusa* (Jones.) leaves in terms of zone of inhibition in mm.(Mean ± SEmean).

Entry	Pathogen	Concentrations (ppm)					
	Fungal/ Bacterial	Water extract			Ethanol extract		
		100	200	500	100	200	500
1.	*Aspergillus flavus*	03.72 ± 0.19[a]	05.56 ± 0.22[a]	13.41 ± 0.25	34.39 ± 0.26	46.49 ± 0.26	63.53 ± 0.20
2.	*Fusarium oxyporum* f.sp-*lini*	11.47 ± 0.17	24.72 ± 0.48	36.38 ± 0.22	70.49 ± 0.27	77.62 ± 0.25	85.31 ± 0.25
3.	*Staphylococcus aureus*	14.31 ± 0.32	29.31 ± 0.22	46.48 ± 0.26	58.27 ± 0.27	79.20 ± 0.34	94.37 ± 0.28
4.	*Streptococcus salivarius*	09.34 ± 0.37	15.22 ± 0.35	28.62 ± 0.21	42.13 ± 0.18	68.29 ± 0.13	76.30 ± 0.38
5.	Control	-	-	-	-		-

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

fungal metabolites are reported to be toxic to man and animals (Matheron *et al*., 2005). The two fungal species viz *Fusarium oxysporum* f sp. *Lini splini* was isolated causing wilt of *Linum ustitatissimun* (linseed) and the other *Aspergillus flavus* was a common aflatoxin producing contaminant of food materials. Aflatoxins are the most notorious of the mycotoxins causing acute and chronic toxicoses in foodstuff (CAST, 2003). The ethanol extract showed very wide zone of inhibition against *Fusarium oxyporum* f.sp-lini (85.31 ± 0.25 mm) and *Staphylococcus aureus* (94.37 ± 0.28 mm) at 500 ppm concentration in comparison to other strains. Water extract was found very less effective against *Aspergillus flavus* (03.72 ± 0.19 mm) at 100 ppm concentration. The extracts showing positive results were found to contain alkaloids (Shaheen *et al*., 2003; Chowdhury *et al*., 2008), flavonoids (Hernandez *et al*., 2000; Mendoza *et al*., 1997), phenols (Akiyama *et al*., 2001), & terpenoids (Amaral *et al*., 1998). In this screening, ethanol extract demonstrated the antimicrobial activity, which support claims related to the topical use of *C. jwarancusa* leaves against dental diseases and also for food preservation.

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. The efficacy of ethanol extracts of *C. jwarancusa* for all the pathogens tested, as well as its higher antioxidant property portends the polarity based potential in biochemicals which are present in the leaves. 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.

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