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IN VITRO ANTIOXIDANT ACTIVITY OF *LAUNAEA PINNATIFIDA* CASS LEAVES

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ABSTRACT
The antioxidant activities of petroleum ether, chloroform, ethanol and water extracts of the leaves of *Launeae pinnatifida Cass* were determined by ferric reducing power, free radical scavenging activity by 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radical scavenging activity. The total amount of phenols and flavonoids were estimated to be 179.46 ± 0.71 mg/g as gallic acid equivalents and 87.46 ± 0.37 mg/g as quercetin equivalents respectively in ethanolic extract. The ethanolic extract exhibited the significant activity against DPPH free radical compared to the other extracts. Ethanol and water extracts gave similar and significant high levels of hydroxyl radical scavenging activities when compared with the standard. Chloroform and petroleum ether extracts showed almost similar results. The results obtained in the present study thus indicate that the ethanolic extract is the potential source of antioxidant.

INTRODUCTION

Reactive oxygen species (ROS) in the form of super oxide anion (O_2^-*), hydrogen peroxide (H_2O_2) and hydroxyl (OH*) are natural byproducts of our body's metabolism. However when present in excess, they can attack biological molecules such as proteins, lipids, enzymes, DNA and RNA, leading to cell or tissue injury associated with degenerative diseases. Although mammalian body has certain defense mechanisms to combat and reduce oxidative damage, epidemiological evidence indicates that the consumption of foodstuffs containing antioxidant polynutrients-notably the flavnoids and other polyphenols is advantageous for health (Amarowicz *et al.,* 2004). The additive and synergistic effects of such bioactive molecules present in plant food are responsible for their potent antioxidant properties (Pereira *et al.,* 2006). Dietary antioxidants provide protection against oxidative attack by decreasing oxygen concentration, by scavenging initial radicals, binding of metal ion catalysts, decomposing primary products of oxidation to non radical compounds, and chain breaking to prevent continuous hydrogen removal from substrates (Subhashinee et al., 2006). In the recent past there has been growing interest in exploiting the biological activities of different ayurvedic medicinal herbs, owing to their natural origin, cost effectiveness and lesser side effects (Naik *et al.,* 2003).

The genus *Launaea* Cass. belongs to the tribe Cichorieae and is represented in India by six species. Of these, *Launaea pinnatifida* Cass. Synonym *L. sarmentosa* (Willd.) Alston is found along the coastal regions from Bengal to ceylone and Madras to Malbar where it serves as sand binders with other plants. It is commonly called as paathri, kneekhowa, almirao, hatarki or hakkarki (Nadkarni, 1982). It is a perennial procumbent herb. The leaflets are simple toothed shape with an average size of 12-15cm long. Leaves have characteristic odour and slightly bitter in taste. It is reported to be tonic, soporifice, and diuretic and used as substitute for taraxacum. Leaves are eaten during famine. Herb is fed to buffaloes as a galactagogue (Kiritikar and Basu, 2001). There is no prior report on the antioxidant activities of *Launaea pinnatifida* cass leaves. In this study the antioxidant activity has been investigated the same through several chemical and biochemical assays.

MATERIALS AND METHODS

Plant materials and extraction

The fresh leaves of *Launaea pinnatifida* Cass (Compositae) were collected from the farmland of Saradagi village, 24 km south of Gulbarga, Karnataka (India) during the flowering month of Oct-Nov 2008. The plant was identified and authenticated. A voucher specimen HGUG/SN-76 is deposited in the department. The leaves were shade dried and powdered to 22-mesh size and then subjected to successive soxhlet extraction with petroleum ether (40º-60ºC), chloroform, ethanol and distilled water until the solvents became colourless. Then the extracts were further evaporated to dryness under vaccume.

Determination of total phenolic components

Total soluble phenolics in the extract were determined with Folin- Ciocalteau reagent (Slinkard and Singleton, 1997) using

gallic acid as the standard phenolic compound.

1 mL of extract solution containing 1g extract in the volumetric flask was diluted with 46 ml of distilled water. 1mL of Folin-Ciocalteau reagent was added and the content of flask was mixed thoroughly. 3 minutes later 3 mL of 2% saturated sodium carbonate was added to the mixture and was allowed to stand for 2 hr with intermittent shaking. The absorbance of the blue color developed was read at 760 nm. The concentration of the total phenols was expressed as mg/g of dry extract (Kim *et al.,* 2003). The concentration of total phenolic compounds in the extract was determined as μg of gallic acid equivalent using an equation obtained from the standard gallic acid curve.

Absorbance = 0.0008 × gallic acid (μg).

Determination of total flavanoids

1mg samples were added to 1mL of 80% ethanol. Aliquot of 0.5mL was added to the test tubes containing 0.1 mL of 10% aluminum nitrate, 0.1 mL of 1 M potassium acetate, and 4.3 mL of 80% ethanol. The absorbance of the supernatant was measured at 415 nm after 40 minutes of incubation at room temperature. Total flavonoid concentration was calculated using quercetin as standard (Nieva Moreno *et al.,* 2000).
Absorbance = 0.002180 × μg quercetin – 0.01089.

Ferric reducing activity

Different concentrations of extracts (20μg, 100μg and 200μg) were mixed with 2.5 mL of 200 mmol/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50ºC for 20 minutes then 2.5 mL of 10% trichloroacetic acid (w/v) was added, the mixture was centrifuged at 1000 rpm for 8 minutes, the upper layer was mixed with 5 mL of deionised water and 1 mL of 0.1% of ferric chloride, and the absorbance was measured spectophotometrically at 700 nm. (Barros *et al.,* 2007). Higher absorbance of the reaction mixture indicated greater reducing power.

DPPH radical scavenging activity

Different concentrations of leaf extracts and Butylated hydroxyl anisole (BHA) (20μg, 100μg and 200μg) were taken and the volume was adjusted to 100μL by adding methanol. 5 mL of 0.1 mM methanolic solution of 1, 2-Diphenyl picryl hydrazine (DPPH) was added. The mixture was shaken vigorously and allowed to stand for 30 minutes in dark. 0.01 mM solution of DPPH in methanol was used as control, whereas BHA was used as a standard. The reduction of DPPH radical was determined by measuring the absorption at 517 nm (Barros *et al.,* 2007). The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation:

$$\% \text{ RSA} = \frac{\text{(Absorbance of control - Absorbance of sample)}}{\text{(Absorbance of control)}} \text{ X } 100.$$

IC_{50} value for the extract *i*.e. the concentration at which it scavenges 50 % of DPPH radical was calculated.

Hydroxyl radical scavenging activity

Different concentrations of leaf extracts and BHA (20 μg, 100μg and 200μg) were taken and the volume was adjusted to 250μL with 0.1 M phosphate buffer (pH 7.4). 1 mL of iron - EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 mL of EDTA (0.018%), and 1 mL of dimethyl sulphoxide (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) were added to these solutions. The reaction was initiated by adding 0.5 mL of 0.22% ascorbic acid. The reaction mixtures were incubated at room temperature for 30 minutes. The reaction was terminated by the addition of 1mL of ice-cold TCA (17.5% w/v). 3mL of Nash reagent was added and the reaction mixture was allowed to stand at room temperature for 15 minutes for colour development. The intensity of the yellow colour formed was measured spectrophotometrically at 412 nm against the reagent blank (Singh *et al.,* 2002). The percentage of hydroxyl radical scavenging activity (HRSA) was calculated by using following equation:

$$\% \text{ HRSA} = \frac{\text{1- (Absorbance of sample)}}{\text{(Absorbance of Blank)}} \text{ X } 100.$$

IC_{50} value of the extract was calculated as mentioned earlier.

Statistics

The experiments were done in triplicate. Data are expressed as mean ± SEM. One- way analysis of variance (ANOVA) was used and the Dunnet's test was used for comparison of mean values. All tests were considered to be statistically significant at $p < 0.01$ by using instant graph-pad software Inc., USA.

RESULTS AND DISCUSSION

There is a strong need for effective antioxidants from natural sources as alternatives to synthetic ones in order to prevent the free radicals implicated diseases (Bravo, 1998). Secondary metabolites such as polyphenols are not required for plant development, but they interact with pathogens, herbivores, and protect the plant from UV radiations and oxidants, repel or poison predators (Winkel-Shirley, 2002). We have determined the amount of phenolic content and flavonoids present in different extracts of *Launaea pinnatifida* Cass leaves (Table 1). Maximum amount of these two were observed in the ethanolic extract *i*.e. 179.46 ± 0.71 mg/g of phenols as gallic acid equivalents and 87.46 ± 0.37 mg/g of flavonoids as quercetin equivalents

Fig.1 represents reductive capabilities of *Launaea pinnatifida* Cass leaves compared to BHA. The reducing power of a compound serves as a basis of an indicator for its potential antioxidant activity (Meir *et al.,* 1995). In our study the reducing power was found to be increased with increased concentration of extract; ethanolic extract exhibited the highest reducing power. Some authors have reported a direct correlation between antioxidant activity and total phenolic content (Velioglu *et al.,* 1998; Ferreira *et al.,* 2007). The antioxidant activity of phenols may be related to their redox properties, which allow them to act as reducing agent or hydrogen atom donors, their ability to chelate metals, inhibit lipoxygenase and scavenge free radicals (Decker, 1997). In the previous table we have reported the phenols and flavonoids. The reducing activity observed here may be due to their ability to act as reducing agent.

Fig. 2 depicts the percentage of free radical scavenging activity of *Launaea pinnatifida* Cass leaves by using DPPH. The later is the stable free radical having the maximum absorption at 517 nm (Kumar *et al.,* 2002) and has been widely utilized to

Table 1: Total phenolic and flavonoid content of various extracts of *Launaea pinnatifida* Cass

Extracts	Total phenols (mg/g) ± SEM	Total flavonoids (mg/g) ± SEM
PE	45.36 ± 0.80	7.78 ± 0.28
CE	62.36 ± 0.45	19.69 ± 0.26
EE	179.46 ± 0.71	87.46 ± 0.37
WE	172.66 ± 0.56	71.54 ± 0.30

PE- Pet ether extract, CE- chloroform extract, EE- ethanolic extract; WE- water extract; Total phenols are expressed as gallic acid equivalent/g; Total flavonoids are expressed as quercetin equivalent/g; SEM = Standard Error Mean

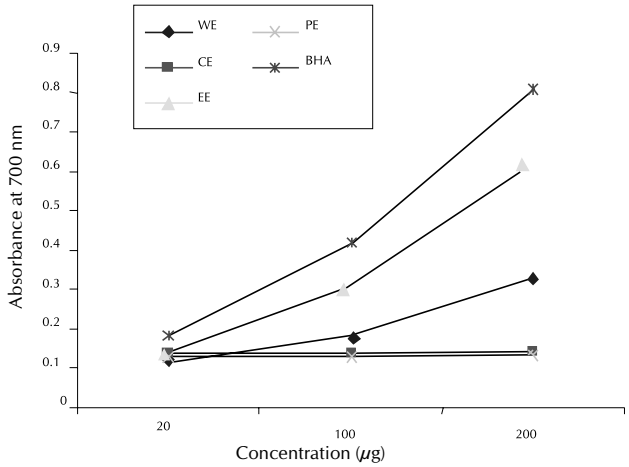

Figure 1: Ferric reducing activity of *Launaea pinnatifida* Cass leaves WE-Water extract, CE- Chloroform extract, EE- Ethanolic extract, PE- Petroleum ether extract, BHA- Butylated hydroxyl anisole

appraise the antioxidant activity of various natural products (Hu and Kitts, 2000). In the presence of compounds capable of donating a hydrogen atom or an electron, its free radical nature is lost; hence a decrease in absorption at 517 nm is seen. Figure illustrates a significant ($p < 0.01$) decrease in DPPH radical due to the scavenging ability of *Launaea pinnatifida* leaves and BHA as reference compound presented highest activity at all concentrations. 200μg/mL of ethanolic extract and BHA exhibited 71.22% and 89.1% inhibition respectively and the EC_{50} values were found to be 609μg/mL and 761.89μg/mL for the ethanolic extract and BHA respectively. The reduction capability of DPPH observed through decrease in

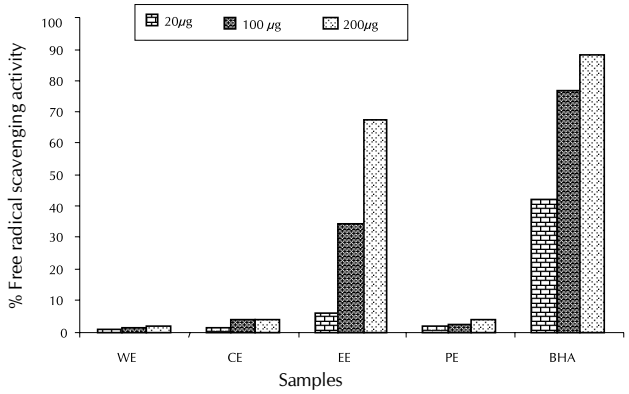

Figure 2: DPPH radical -scavenging activity of *Launaea pinnatifida* Cass leaves; SWE-Water extract, CE- Chloroform extract, EE- Ethanolic extract, PE- Petroleum ether extract, BHA- Butylated hydroxyl anisole

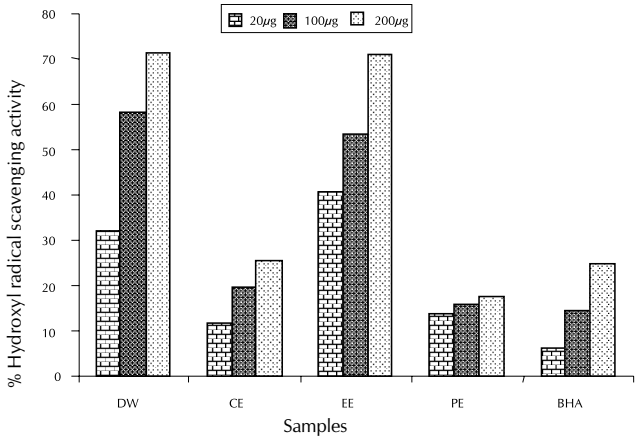

Figure 3: Hydroxyl radical scavenging activity of *Launaea pinnatifida* Cass leaves; WE-Water extract, CE- Chloroform extract, EE- Ethanolic extract, PE- Petroleum ether extract, BHA- Butylated hydroxyl anisole

its absorbance in our studies may be due to inhibited concentration of free radicals.

Fig. 3 shows the percentage of hydroxyl radical scavenging activity of *Launaea pinnatifida* Cass leaves. Hydroxyl radical is the most reactive among ROS and it bears the shortest half-life compared with other ROS. In this study, administration of leaf extracts to the reaction mixture significantly inhibited the hydroxyl radical activity; maximum inhibition of 72.46 and 71.23 being observed with water and ethanol respectively. This inhibition was quite more when compared with the standard (24.26%). The chloroform extract also exhibited slightly higher percentage of inhibition (26.10 %) when compared with the standard. It means that the test extracts can act as better hydroxyl radical scavenger than the standard. These results were found statistically significant ($p < 0.01$). The EC_{50} values for distilled water, ethanol, chloroform and pet ether extracts were found to be 597μg/mL, 587μg/mL, 215μg/mL and 149.38μg/mL respectively.

As observed from the data the components present in the extracts of *Launaea pinnatifida* Cass have high antioxidant activity and its various antioxidant mechanisms may be attributed to the strong reducing power and its effectiveness as a good scavenger of free radicals. According to many reports, a highly positive relationship between total phenols and antioxidant activity has been found in extracts of many plant species. Naturally occurring Phenolic compounds are reported to possess free radical scavenging properties, due to their hydroxyl groups (Diplock, 1997). It is apparent from the present study that the components present in leaves not only scavengers off the free radical but also inhibits the generation of free radicals. Further, phenols are effective hydrogen donors, which make them antioxidant (Rice-evans, *et al.,* 1995). It may be thus concluded that the fractions obtained from various extracts of *Launaea pinnatifida* Cass leaves possess significant antioxidant activity. The antioxidant potential may be attributed to the presence of phenolic compounds; further *in vivo* studies are in progress.

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