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RESEARCH ARTICLE

Identification of Total Phenolics, Total Flavonoids and Antioxidant Potential in the Leaf Extracts of *Launaea procumbens* Roxb

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ABSTRACT

The present study is based on the aim to determine the possible use of plant *Launaea procumbens* in the field of therapeutics for oxidative stress and cancer studies. In the recent years, a trend for studies on oxidative stress has been remarkably raised. Currently used, synthetic antioxidants possess several side effects and also are suspected to have been promoting negative health effects. Therefore restrictions have been placed on their application, and curiosity is generated to substitute them with naturally occurring antioxidants. Hence, in the present study leaves of the plant *Launaea procumbens* were evaluated for the total phenolics, total flavonoids and antioxidant potential by DPPH radical scavenging assay. *In vitro* experimental assays on plant extract viz., Folin – Ciocalteus colorimetric method, Aluminum Chloride colorimetric method and DPPH radical scavenging assay were used with analysis being done on Graph-pad Prism Software for biological data. The concentration of total phenolics in test sample is calculated from the standard graph of quercetin and found to be 345 mg quercetin equivalent/gm of fresh mass, while the total flavonoids was calculated to be as 11.56 mg quercetin equivalent/gm of fresh mass. The antioxidant potential of the plant was calculated by % inhibition of the DPPH and found to be 200 µg/ml for >90 % inhibition with the IC_{50} value of 147.1. The results from the present studies revealed that the plant *Launaea procumbens* show marked scavenging potential with appreciable amount of total phenolics and total flavonoids.

KEYWORDS

Total phenolics, Total flavonoids, Antioxidant potential, DPPH radical scavenging, Quercetin standard and Launaea procumbens

INTRODUCTION

There is a number of reactive oxygen species, commonly called as ROS, produce from the exogenous factors or from several biological reactions, as bi-products.

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These ROS can be categorized as superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide.[1] ROS produced in the biological processes are generally metabolized in other biological reactions or can be utilized for the phagocytosis or intercellular signaling. ROS produced due to the exogenous factors such as UV radiation, ionization therapy, chemical reactions and

metabolic wastes, which cannot be degraded further, have a wide variety of pathological effects generally including DNA damage, carcinogenesis and various degenerative disorders such as cardiovascular diseases, aging or neuro-degenerative diseases.[2,3,4]

With the advancement in the life sciences, it has been conceptualize that the free radicals or oxidative injury appears to be the fundamental mechanism underlying a number of human neurologic and other disorders.[5,6] In carcinogenesis, ROS are responsible for initiating the multistage carcinogenesis process starting with DNA damage and accumulation of genetic events in one or few cell lines which leads to progressively dysplastic cellular appearance, deregulated cell growth, and finally carcinoma.[7] Hence, therapy using free-radical scavengers (antioxidants) has potential to prevent, delay or ameliorate many of these disorders.[8]

Currently used, synthetic antioxidants are having several side effects and also suspected to have been promoting negative health effects[9], hence restrictions has been placed on their applications and curiosity is generated to substitute them with naturally occurring antioxidants. Besides the well-known and traditionally used natural antioxidants such as tea, wine, fruits, vegetables and spices[10], many other plant species have been investigated in the search for novel antioxidants.[11] There are also some naturally produced antioxidants which are already in use for the nutritional supplements or are commercially exploited as the antioxidant additives.[12]

Recent studies have shown that a number of plant products including polyphenols, terpenes and various plant extracts exerted an antioxidant action.[13] It has been hypothesized and documented that the fruits and vegetables constitute a large amount of these secondary metabolites and also there is a considerable amount of evidence revealing an association between individuals who have a diet rich in fresh fruits and vegetables and the decreased risk of cardiovascular diseases and certain forms of cancer.[14,15] New potato cultivars with purple colored flesh of tubers and high antioxidant activity are recently investigated for improving human diet for healthier life.[16] Hence, there is currently an immense interest in exploiting the natural antioxidants and their role in human health and nutrition.[17,18] These studies made the authors to analyze the plant *Launaea procumbens* Roxb., for its phenolic constituents and flavonoids. The antioxidant potential of the plant leaves is also determined using the most acceptable method of DPPH scavenging.[19,20]

The genus *Launaea* belongs to the family *Asteraceae*. The plants in this genus are perennial to pauciennial herbs, small rosette shrubs, subshrubs, spinescent shrubs or annuals, all tap rooted and roots often shoot bearing. The leaves are sessile and commonly rosette, at least in juvenile plants. *Launaea procumbens* is a polymorphic perennial herb with shoot bearing roots (therefore plants often growing in groups), flowering up to 20-40 cm high, with basal leaf rosette and with (one to) several, rather week, procumbent to ascending-erect, leafy to leafless flowering stems; aging plants with woody (and often branched) base; rosulate leafy innovations at lower nodes of the stems often present.

MATERIALS AND METHOD

Collection of Samples

Fresh plants were collected from, Shree Bapalal Vaidhya Botanical Graden, located in the Veer Narmad South Gujarat University Campus, Udhna Magdalla Road, Surat, Gujarat, India. Taxonomic identity of the plant was confirmed by the Taxonomists in Department of Biosciences, Veer Narmad South Gujarat University and the specimen voucher collection were preserved in the herbarium of the Department. The leaves from the plants were separated, washed under the running tap water and dried at 45°C in the oven. The dried leaves were

then homogenized to fine powder and stored in the air tight container for future use.[21]

Chemicals and Reagents

All the solvents used were of HPLC grade and procured from Merck, Germany. The standard, Quercetin, and the chemical such as 2, 2 – diphenyl picryl hydrazyl (DPPH) were procured from the Sigma Aldrich, St. Louis, MO, USA.

Preparation of Extracts

Methanolic solvent extract was prepared by, adding 10 gm of plant powder to 100 ml of the solvent. The solution was then heated at 55°C on water bath for about 5 min and then sealed with the glass stopper and kept on the rotary shaker for 24 hrs. After 24 hrs, the solution was concentrated under reduced pressure at 45°C using the rotary evaporator to $1/10^{th}$ of the initial volume.[21]

Determination of Total Phenolics

Determination of the total phenolics in the leaf extract was done according to the Folin – Ciocalteus colorimetric method.[22,23] A working quercetin standard of 200 µg/ml in the methanol was prepared and used as the reference standard. For the estimation of total phenolics, six test tubes, prewashed with acid and distilled water, were taken, and to each tube 0.0 ml, 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1.0 ml of the working standard solution are dispensed. The final concentration of working standard in each tube was prepared to 0.0 µg/ml, 40 µg/ml, 80 µg/ml, 120 µg/ml, 160 µg/ml and 200 µg/ml by phosphate buffer at pH 7.0. To each of the tube immediately 4 ml of the 1 N Folin – Ciocalteus reagent was added followed by 5 ml of 7.5 % Sodium Carbonate. The tubes were then incubated for 60 min in dark at room temperature. After 45 min, absorbency of each tube was recorded at 765 nm and the standard graph was plotted for absorbency against concentration. The same method is adopted for the preparation of test sample, where 100 µl (i.e. 0.1 g) of the prepared plant extract was taken in a separate test tube containing 900 µl of the phosphate buffer at pH 7.0 (make the final volume 1 ml). To the mixture 4 ml of the 1 N Folin – Ciocalteus reagent was added followed by the addition of 5 ml of 7.5 % Sodium Carbonate. The test mixture was then kept in dark for 60 min at room temperature and the absorbency was recorded at 765 nm. The concentration was calculated by comparing absorbency from the standard graph.

Determination of Total Flavonoids

Determination of the total flavonoids in the leaf extract was done based upon the Aluminum Chloride Colorimetric method.[24] A 500 µl (i.e., 0.5 g) of the prepared extract was mixed with the 500 µl of the methanol, making up the final concentration 500 µg/ml. For the estimation of total flavonoids, 1 ml solution of the prepared extract of 500 µg/ml concentrations was taken in 10 ml volumetric flask, prewashed with acid and distilled water. To this solution, 0.3 ml of 5 % $NaNO_2$ was added followed by the addition of 0.3 ml of 10 % $AlCl_3$ solution, after 5 min of incubation. After the addition of $AlCl_3$, 2 ml of 1 M NaOH was immediately added followed by the addition of distilled water to make the final volume 10 ml. The content was mixed well by shaking the flask gently for at least 2 min and the absorbency was taken at 510 nm against the prepared reagent blank. The absorbency is reported as the % concentration equivalent to 100 gm of fresh mass.

Determination of Antioxidant Potential

The antioxidant potential of the plant leaf extract was determined by the DPPH radical scavenging activity[19,20]. For the estimation process, six test tubes, prewashed with acid and distilled water, were taken, and to each tube 0.0 ml, 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1.0 ml of the 200 µg/ml of the extract, prepared in methanol, were added. The final concentration of the sample extract in each

tube was prepared to 0.0 µg/ml, 40 µg/ml, 80 µg/ml, 120 µg/ml, 160 µg/ml and 200 µg/ml with methanol. From each tube, 0.75 ml of the solution is taken and transferred to another tubes, prewashed with acid and distilled water. To this solution, 1.5 ml of DPPH methanolic solution is added in each tube respectively. The tubes were then incubated in dark for 20 min at 30°C and then the absorbance were taken at 517 nm. The calculation of % inhibition or % scavenging was calculated as follows. The same procedure was followed with the standard Quercetin solution for positive control and blank reagent.

$$\%\text{Inhibition} = \left[\frac{\text{Absorbance of blank} - \text{Absorbance of test}}{\text{Absorbance of blank}}\right] \text{X } 100$$

Determination of IC_{50}: Many log (inhibitor) vs. response curves follows the familiar symmetrical sigmoidal shape. The goal is to determine the IC_{50} of the inhibitor – the concentration that provokes a response half way between the maximal (top) response and the maximally inhibited (bottom) response. Therefore the data generated from assay of DPPH scavenging activity were plotted in the Graphpad Prism (v 6.0.1.0) software and the IC_{50} value for the extract was determined.

RESULTS

Determination of Total Flavonoids

The total flavonoids in the methanolic leaf extract of the *Launaea procumbens* were calculated to be as mg quercetin equivalent (mg quercetin/gm extract) and found to be 11.56 mg quercetin equivalent/gm of the fresh mass, whereas the percentage concentration of total flavonoids in the extract was calculated as 0.23 %.

Determination of Total Phenolics

From the plotted standard graph, Fig 2, the concentration of total phenolics in the methanolic leaf extracts of *Launaea procumbens* were calculated and found to be *345 mg* quercetin equivalent/gm of fresh mass. The percentage concentration of the total phenolics in the extract were calculated from the observed value of total phenolics and found to be *17.25 %*.

Determination of Antioxidant Potential

The absorbance of the standard quercetin and different concentration of prepared extracts were taken at 517 nm, and from the absorbance, % inhibition of the extract was calculated. The result for the % inhibition and absorbance of the extract and the standard quercetin are shown in the Table 1 and 2. The graph plotted from the result table for absorbance at 517nm and % inhibition of DPPH depicts the linear increase in the inhibition of free radical. The figure 3 represents the graph for % inhibition of DPPH by the crude methanolic extract and figure 4 represents the % inhibition of DPPH by standard quercetin.

Determination of IC_{50} Value

The IC_{50} value of %inhibition was calculated for extract with the help of Graphpad Prism (v 6.0.1.0) software and found to be 147.1 (Fig.4 & 5), whereas the IC_{50} value of standard quercetin was found to be 31.61 (Fig 5 & 6). The values of the IC_{50} are also plotted in the Table 1 and 2.

DISCUSSION AND CONCLUSION

Some secondary metabolites are considered as metabolic waste products, for example, alkaloids may function as nitrogen waste products. However, a significant portion of the products derived from secondary pathways serve either as protective agents against pathogens (e.g. insects, fungi or bacteria) or growth regulatory molecules (e.g. hormone-like substances that stimulate or inhibit cell division and morphogenesis). Due to these physiological functions, secondary metabolites are potential anticancer drugs, since either direct cytotoxicity is effected on cancer cells or the course of tumor development is modulated, and eventually inhibited. Administration of these compounds at low concentration may be lethal for microorganisms and small animals,

Table 1: Absorbance of Launaea procumbens at 517 nm and % inhibition of DPPH

Sr. No.	Sample Concentration	Absorbance	% Inhibition	IC_{50}	$LogIC_{50}$
1	0µl/ml (Blank)	1.098	0	147.1	2.168
2	40µl/ml of Extract	0.848	22.76		
3	80µl/ml of Extract	0.587	46.53		
4	120µl/ml of Extract	0.394	64.11		
5	160µl/ml of Extract	0.185	83.15		
6	200µl/ml of Extract	0.103	90.61		

Table 2: Absorbance of quercetin at 517nm and % Inhibition of DPPH

Sr. No.	Sample Concentration	Absorbance	% Inhibition	IC_{50}	$LogIC_{50}$
1	0µg/ml Quercetin	1.098	0	31.61	1.500
2	5µg/ml Quercetin	0.838	23.67		
3	10µg/ml Quercetin	0.648	40.98		
4	15µg/ml Quercetin	0.494	55.00		
5	20µg/ml Quercetin	0.241	78.05		
6	25µg/ml Quercetin	0.103	90.61		
7.	30µg/ml Quercetin	0.064	94.17		

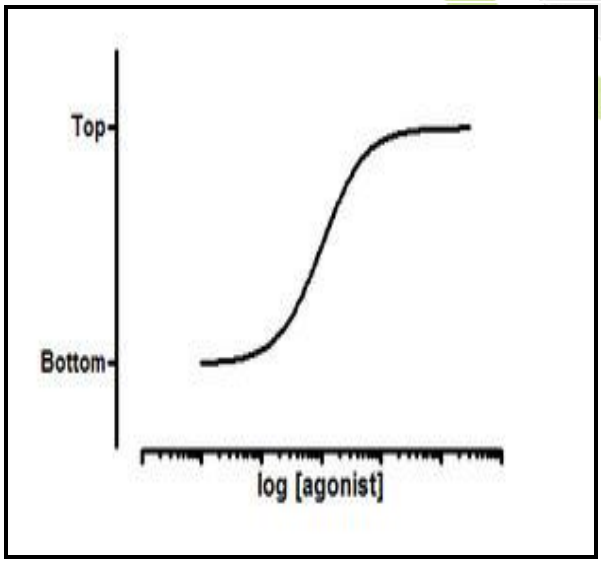

Figure 1: Model equation of log (inhibitor) vs. response – variable slope

Y=Bottom + (Top-Bottom) / (1+10^ ((LogIC$_{50}$ – x) * HillSlope))

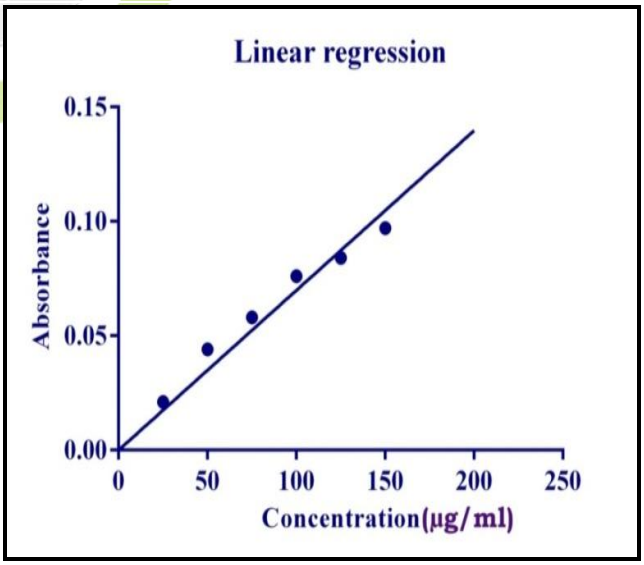

Figure 2: Standard graph of linear regression for standard quercetin at 765 nm

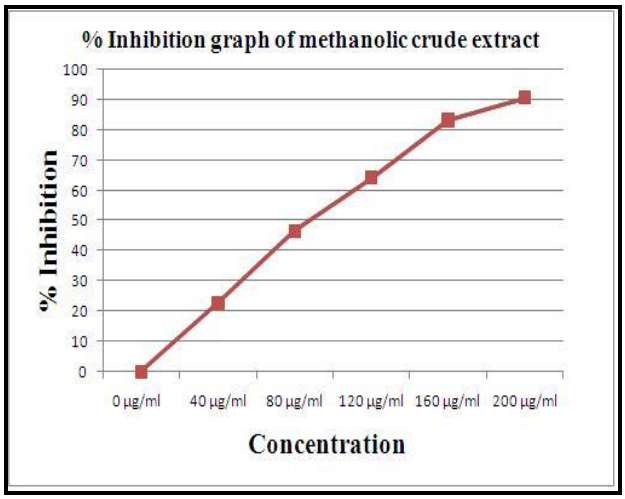

Figure 3: % Inhibition graph of methanolic crude extract of *Launaea procumbens* for DPPH

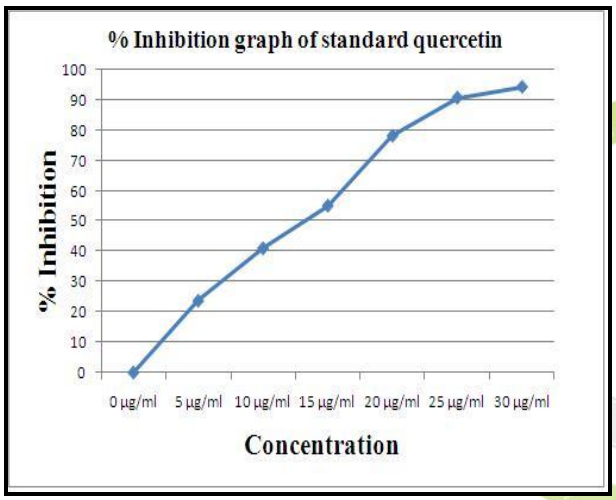

Figure 4: % Inhibition graph of standard quercetin for DPPH

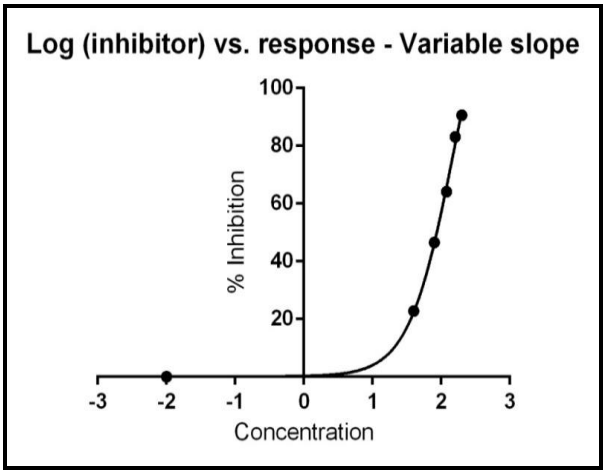

Figure 5: Log (inhibitor) vs. response – Variable slope for methanolic crude extract of *Launaea procumbens* for determination of IC_{50} values.

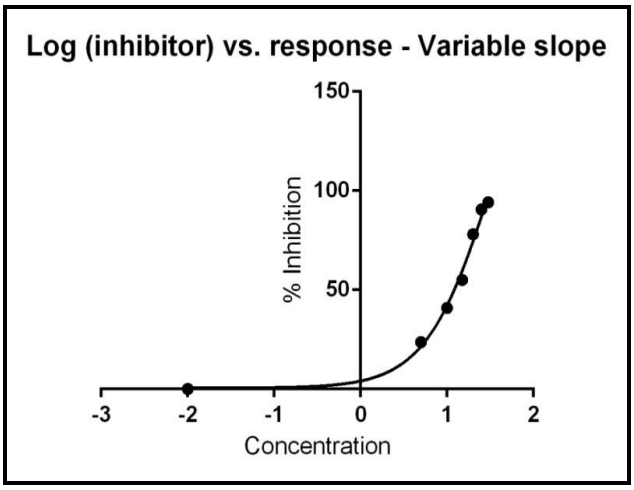

Figure 6: Log (inhibitor) vs. response – Variable slope of quercetin for determination of IC_{50} values.

such as herbivorous insects, but in larger organisms, including humans, they may specifically affect the fastest growing tissues such as tumors.[23]

The results from the present studies revealed that the plant *Launaea procumbens* show marked scavenging potential. The results are also in similarity with the investigation of Hagerman et al., (1998) and Falleh et al., (2008), who reported that medicinal plants markedly scavenge free radicals. The antioxidant potential of the methanolic extract of *Launaea procumbens* could be due to the presence of plant bioactive phenolic and polyphenolic compounds which significantly reduce the free radicals which cause oxidative stress. The results from the studies on identification of total flavonoids and total phenolics in the extract are in support of this hypothesis.

Although, the IC_{50} value of extract shows a comparatively higher concentration than quercetin, it cannot be overlooked to the possibility of antioxidant potential of the plant. The increase in IC_{50} value may be due to several other compounds hindering the effect to be solely potent. Hence for the same, pure compounds from the plant may be extracted, isolated and evaluated for the true potential of plant in scavenging of the free radicals as this could surely benefit the

search of the new compounds for anticancer properties.

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