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PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITIES IN DIFFERENT SOLVENT EXTRACTS OF *LAUNEAEA PROCUMBENS*

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ABSTRACT

The main objective of present study were screening of phytoconstituent produce by *Launaea procumbens* and evaluate it phytochemically by using four different solvents(petroleum ether, acetone, methanol and aqueous) in terms of total phenolic content (TPC), total flavonoid content (TFC) and antioxidant properties analysis. Antioxidant potential of the extracts was evaluated by non-enzymatic assays (DPPH, ABTS). Lowest IC_{50} values were shown by methanolic extracts followed by acetone, aqueous and petroleum ether extracts. The antioxidant properties of the extracts showed positive correlation with the total phenolic and flavonoid content of the extracts. The finding indicated that some of the recognized phytochemicals possess pharmacological actions which may be responsible for its medicinal values.
Keywords: *Launaea procumbens*, total phenolic content, total flavonoid content, antioxidant properties.

Introduction

Medicinal plants except drugs are also a huge source of knowledge and information for a wide variety of phytoconstituents which could be developed as drugs with specific bioactivity. These are the natural reservoirs of essential and active chemical compounds such as alkaloids, glycosides, oils, resins, steroids, tannins, phenols and flavonoids which are stored in their specific parts such as stem, leaves, flowers, fruits, bark, seeds and roots (Vijayalakshmi and Ravindran, 2012; Doss, 2009). The medicinal effects of plant materials typically result from the mixture of these secondary products (Rani, 2019). Correlation between the medicinal values and phytoconstituents of plant is necessary to know for the compounds synthesis with specific bioactivities to cure different chronic diseases (Pandey *et al.,* 2013). Traditional folk remedies from wild plants have always direct scientists to search for new drugs to provide healthy life for animals and humans (Kapoor *et al.,* 2021). In addition, few medicinal plants are still hidden within the plant which needs to be scientifically evaluated.

Genus *Launaea* comprises of 40 different species and belongs to the Asteraceae family. *Launeaea procumbens* (Roxb.) is a perennial herb commonly known as Creeping Launeae and Junglibooti shown in Figure- 1. It is broadly distributed in different parts of India and used broadly in herbal, ayurvedic and traditional medicine system (Ali *et al.,* 2003). Traditionally, it has been used in the treatment of kidney disorder, Gonorrhea, Jaundice, Rheumatism and liver disorders (Ahmad *et al.,* 2006). Previous studies revealed the presence of different phytoconstituents in Launaea genus like lupeols, phenols, triterpenes, sesquiterpene lactones, steroids, and flavonoids (Yadava and Chakravarti, 2009; Moussaoui *et al.,* 2010). Based on the study of literature and its medicinal importance, the present study was planned to evaluate the different extracts of *L. procumbens* for its antioxidant properties with regards to various phytoconstituents and total phenolic and flavonoid contents.

Fig. 1 : *Launeaea procumbens* Plant

Materials and Methods

Collection of Plant

The selected plant collected from waste places and road sides in Feb-March 2019. Plant materials were washed with running tap water to remove dust and finally with distilled water.

Sample Preparation

Plant materials were completely dried under shade at room temperature and then crushed to obtain a fine powder. 50 g powdered plant material was extracted (1:5W/V) in a Soxhlet apparatus in methanol, acetone, aqueous and petroleum ether till the solution become transparent. The extracts were filtered through a whatman paper and then concentrated by using a rotary evaporator at room temperature. Then these extracts were used for further analysis. % yield of extract was calculated using following formula:

% yield= weight of crude extract (g)/ total weight of plant powder (g) X 100

Preliminary Phytochemical Screening

The prepared extracts were analysed for the occurrence of different bioactive phyto-compounds as per standard approaches recorded in Table-1.

Table 1: Standard methods for preliminary phytochemical analysis:

Bioactive phytocompounds	Test	Procedure	Observation	References
Alkaloid	Mayer's Test	Few drops of Mayer's reagent (Potassium Mercuric Iodide) added into To 1 mL Plant extract.	yellow coloured precipitate formed	(Harbone, 1998)
Amino-acid	Ninhydrin test	Add few drop of 1% ninhydrin solution into 2 mL of Extract.	Purple colouration	(Onwukaeme *et al*., 2007)
Flavonoids	Alkaline Reagent Test	In 2 mL of Plant extract was treated add 3 ml of dilute NaOH, tariled by addition of 3-4 mL dilute HCl.	Formation of intense yellow color, which becomes disappear on addition of dilute HCl.	(Hossain *et al*., 2015)
Phenols	Ferric Chloride Test	4-5 drops of ferric chloride added into 2 mL of Plant extract, mix well.	Dark green color appear indicates the phenol presence	(Kabesh *et al*., 2015)
Tannins	Braemer's test	1 mL of 10% alcoholic ferric chloride was added to 2-3 mL of plant extract.	Formation of dark blue or greenish grey colour of the solution	(Kala *et al*., 2011)
Terpenoids		1 mL of Plant extract was dissolved in 2 mL of methanol and then vaporized to dryness trailed by the addition of 3 mL of Conc. H_2SO4.	Creation of reddish brown color.	(Aiyegroro and Okoh, 2010)
Saponins	Foam test	2 mL Plant extract, was mixed with 5 mL distilled water in a test tube and shaken vigoursly.	Stable foam appear at the top of solution	(Mir *et al*., 2013)

Total Phenolic Content (TPC)

Total phenolic content was measured by Folin-Ciocalteu method (Onwukaeme *et al*., 2007). 1 mg/mL solution of each plant extract, 5 mL of Folin-Ciocalteu and 2mL of Na_2CO_3 was added. The solution was mixed and incubated for 15 minutes in dark. Absorbance was measured at 620nm. Tannic acid was used as standard. Result was expressed in terms of mg of tannic acid equivalent (GAE)/ gram dry weight of sample. All tests were performed in triplicates.

Total Flavonoid Content (TFC)

Total flavonoid content was estimated by Aluminium Chloride Colorimetric method (Rani *et al*., 2020). 3 mL of each extract, 0.2 mL of 10% aluminium chloride, 0.2 mL of 1M potassium acetate and 5.6 mL of distilled water was added. The mixture was mixed properly and left at room temperature for half an hour. The absorbance was taken at 420 nm. Quercetin was used as standard. All tests were performed in triplicates and results were expressed as mg of quercetin equivalent (QE)/ gram dry weight of sample.

Antioxidant activities

DPPH Assay

The free radical scavenging activity of plant extracts was measured using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay (Blois-1958). 1 mg/mL stock solution of plant extract in their respective solvents was diluted from 10-100 μg/mL. 1 mL of 0.3 mM DPPH solution was added to 1 mL of the plant extracts of different concentrations and also to the ascorbic acid standard. Samples were incubated for 30 minutes in dark. The absorbance was measured at 517 nm. Three biological mean were recorded. Antioxidant activity was calculated by using the formula given below:

% Inhibition = (Absorbance of control–Absorbance of sample)/ Absorbance of control× 100

ABTS Assay

ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) assay was also used to carry out the antioxidant potential of plant extracts as per method described by (Shirwaikar *et al*., 2006). ABTS radical (ABTS+) was generated by reacting 7 mM ABTS solution with 2.45 mM ammonium per sulphate and allowing the mixture to stand at room temperature for 12-16 h in dark before use. The ABTS solution was diluted with ethanol to get an absorbance of 0.700 at 745 nm. A 1 mg/mL stock solution of each plant extract and standard in their respective solvents and diluted from 10-100 μg/mL. 1 mL solution of ABTS was added to the 1 mL of plant extracts of different concentration. The absorbance was measured at 745 nm. Antioxidant activity was calculated as below:

% Inhibition = (Absorbance of control – Absorbance of sample)/ Absorbance of control× 100

Results and Discussion

Percentage yield

Yield % of the different solvent extracts was calculated and the results are given in Table-2, finding publicized that the maximum % yield was observed in methanol extract (9.8 %), trailed by acetone extract (7.7 %), aqueous extract (6.1 %) while minimum yield was obtained in petroleum ether extract (3.3 %).

Table 2 : Percentage yield of the leaves extracts of *Launeaea procumbens* prepared in different solvents:

Sr. No.	Solvent	Method of extraction	Physical nature	Colour	% yield
1	Methanol	Soxhlet apparatus	Solid	Brownish green	9.8
2	Acetone		Solid	Greenish brown	7.7
3	Aqueous		Semi-solid	Brownish	6.1
4	Petroleum ether		Solid	Light green	3.3

Phytochemical Analysis

The plant extracts of *L. procumbens* prepared in four solvents were assessed for the occurrence of different phytochemicals and the result obtained are presented in Table- 3, perusal of the results reveal that all the tested extracts, showed the presence of alkaloids, phenols and flavonoids. Methanolic extract showed the maximum number of phytoconstituents.

Table 3 : Phytochemical analysis of plant extracts of *L. procumbens* prepared in different solvents.

Phytochemicals	Plant extracts			
	Methanol	Acetone	Aqueous	Petroleum ether
Alkaloid	+	+	+	+
Amino-acid	+	+	-	-
Flavonoids	+	+	+	-
Phenols	+	+	+	-
Tannins	+	+	-	-
Terpenoids	+	-	-	-
Saponins	-	-	-	-

** (+) indicate the presence, (-) indicate the absence of phytoconstituents.

Total Phenolic and Flavonoid Content

Maximum TPC and TFC was found in methanolic extract (59.53±0.66; 48.08±0.16) followed by acetonic extract shown in figure-2 and 3. The least value of TPC (21.19± 0.19) and TFC (15.36 ± 0.31) was found in case of petroleum ether extract. Similar to this finding, (Khan-2017; Adinortey *et al*-2018) also reported maximum total phenolic and flavonoid content in methanolic extract of *L. procumbens*.

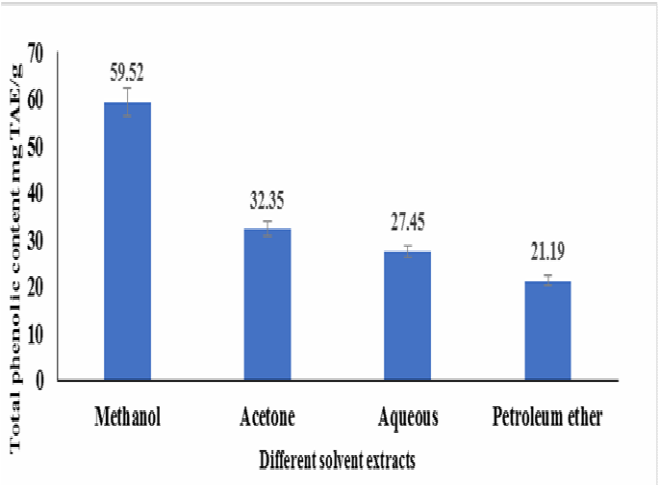

Fig. 2 : Total phenolic in different solvent extracts of *L. procumbens*

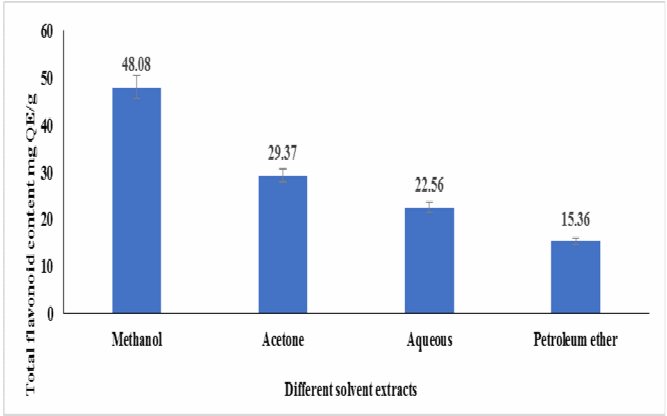

Fig. 3 : Total flavonoid content in different solvent extracts of *L. procumbens*

DPPH Assay

The maximum antioxidant activity was found in the methanolic extract showing the least IC-50 value *i.e.* 39.56 ± 0.07 followed by acetonic extract (74.25± 0.28) and poorest activity was shown by the petroleum ether extract having maximum IC-50 value (89.94± 0.42) Figure 4 and 5.

Maximum antioxidant activity in methanolic extract might be due to the occurrence of large amount of phenolic and flavonoid compounds. Phenolic compound possesses free hydroxyl group which is responsible for scavenging

activity. Methanolic extract of *L. procumbens* is found more potent as compared to the other solvent extracts and found similar with some previous reports reported by (Khan *et a.,* 2010). On a similar pattern our results also showed that highest TPC and TFC are present in methanolic extract. On a similar pattern of antioxidant potential was also least in case of petroleum ether.

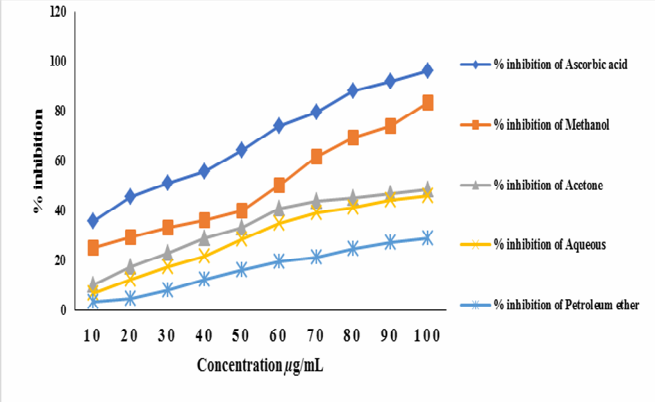

Fig. 4: Inhibition Percentage of DPPH radical by plant extracts of *L.procumbens*

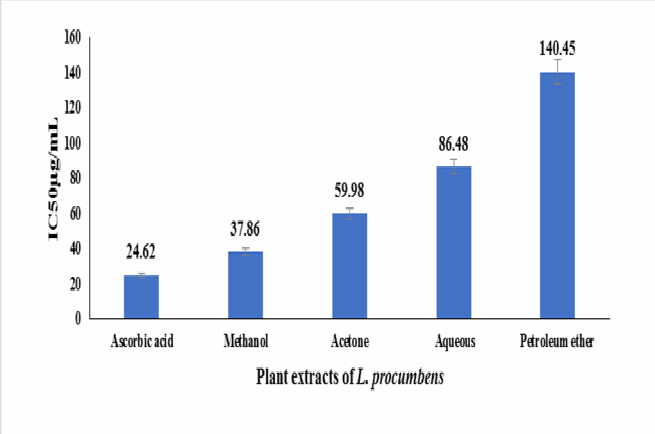

Fig. 5 : IC-50 values of plant extracts.

ABTS assay

On a similar pattern of DPPH assay results, antioxidant activities of the extracts towards ABTS radical was also maximum with the methanolic extract showing lowest IC-50 value (73.22± 0.22) followed by acetonic extract (95.97± 0.48) and lowest in case of petroleum ether extract (147.12± 0.23) figure 6 and 7. Our results agree with a previous report of reported by (Khan *et al*., 2012).

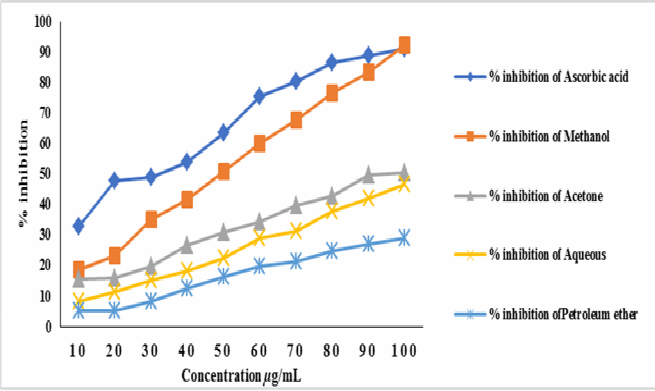

Fig. 6 : Inhibition Percentage of ABTS radical by plant extracts of *L. procumbens*

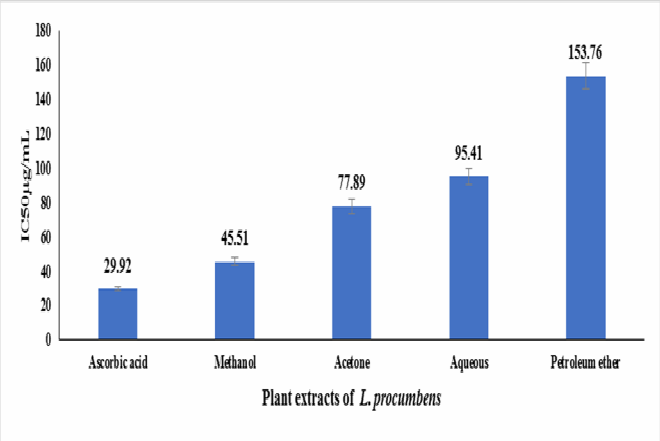

Fig. 7 : IC50 values of plant extracts.

Conclusion

The outcome obtained in the current study has important value with respect to the antioxidant potential especially of methanolic extract. The presence antioxidant activities may be because of the presence of high amount of flavonoids present in methanolic extract. Further, current study suggested that the methanolic extract can be used as a safe and effective source of antioxidants, as an ethnomedicine and on a marketable basis for the development of new drugs.

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