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Short communication

Quantitative estimation of total phenols in *Calyptocarpus vialis* - An emerging weed in Karnataka

Kavitha Sagar*
Botanical Garden & Herbarium, UAS, GKVK, Bengaluru, Karnataka 560 065

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Calyptocarpus vialis Less. (Asteraceae) commonly known as straggler daisy, has already become aggressive invasive species in Karnataka (Rao and Sagar 2012) in Mysuru, Bengaluru, Ballari, Dharwad, Hubballi, Vijayapura, Kalaburagi (South India). It is now spreading rapidly in other places in Karnataka. Due to its monoculture thickets, associated floras have been suppressed. This directly points towards its allelochemical released into the environment. Present study was carried out in order to quantify the total phenols which are responsible for the invasive and aggressive nature of *C. vialis*.

C. vialis whole plants were collected, washed to remove mud and other dust particles. The leaves, stem and roots were separated and dried under shade. The dried samples were crushed separately using blender and the powder thus obtained was stored in air tight container. Twenty gram of each dried sample was dissolved in 200 ml of methanol, chloroform, ethyl acetate in conical flask and kept on rotary shaker for 24 h then filtered and centrifuged at 5000 rpm for 15 min. Supernatant was collected and the solvent was evaporated in watch glass. The dried extract was collected in airtight vials and stored at 4 °C for further studies. Aqueous extract was prepared by boiling 20 g of plant powder in 200 ml distilled water on heating mantle for 30 minutes. The extract was filtered, evaporated, concentrated and preserved at 4 °C. Phytochemical analysis was done following standard methods (Sadasivam and Manickam 1996).

Molisch's test was performed for carbohydrate estimation. Two ml of extract, 3-4 drops of Molisch's reagent was added and mixed properly. To this, concentrated sulphuric acid was added along with walls of the test tube. Appearance of a purple or blue ring in between the two layers indicates the presence of carbohydrate.

Biuret test was performed for protein. Two ml of biuret reagent (mixture of 2 ml of 10% NaOH and 2-3 drops of 0.5% $CuSO_4$) was added to the crude extract and heated. Appearance of purple/blue color confirms the presence of proteins.

Mayer's test was done for alkaloid estimation. Crude extracts were evaporated to dryness and residues were heated with 2% Hydrochloric acid on a boiling water bath. The extract were cooled, filtered and treated with the Mayer's reagent. Presence of yellow precipitation or turbidity shows the presence of alkaloids.

Ferric chloride test was done for phenol. Two ml of plant extract, 2ml of distilled water followed by 10 % $FeCl_3$ solution was added. Bluish black colour indicates the presence of phenol and tannins.

Foam test was done for saponin estimation. Two ml of extract was taken in a test tube and 10 ml of distilled water was added and shaken vigorously. Formation of foams confirms the presence of saponin.

Gelatin test was done for tannins. Crude plant extracts were treated with 5 ml of 1% gelatin solution containing NaCl and observed for the occurrence of white precipitate.

Flavonoid were estimated by adding 2 ml of 50% methanol in 4 ml of extract. The solution was warmed and metal magnesium was added. This was followed by addition of 5 to 6 drops of concentrated hydrochloric acid. Red coloration confirms the presence of flavanoids.

For determination of glycosides, any one of tests given below was done. Libermann's test was done for glycosides. Two ml of chloroform and 2 ml of acetic acid was added. The solution was ice cooled followed by addition of concentrated H_2SO_4. Colour change from blue to green indicated the presence of glycosides; Salkowski's test was done crude extract was dissolved in 2 ml of chloroform. To this conc. H_2SO_4 was added and the mixture was shaken. Formation of reddish brown color indicates the presence of glycosides; Keller-kilani test to the crude extract was added 2 ml of acetic acid and few drops of 2% $FeCl_3$ solution. The entire mixture was then

***Corresponding author:** kavcsa@gmail.com

poured in a test tube containing 2 ml of conc. H_2SO_4. A brown ring at the junction indicates the presence of glycoside.

Sulphuric acid test was done for steroids. Two ml of chloroform, 2 ml of conc. H_2SO_4 was added by the sides of the test tube and observed for red color at the lower chloroform layer.

Sulphuric acid test was done for terpenoids. Crude plant extract was dissolved in 3 ml of chloroform. This was than evaporated to dryness and 2 ml of conc. H_2SO_4 was added and heated for about 3 minutes. A grayish colour indicated the presence of terpenoids.

Ethyl acetate, chloroform and aqueous plant extracts was determined following method of Singleton *et al.* (1999). The reaction mixture was prepared by mixing 0.5 ml of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in distilled water and 2.5 ml 20% Na_2CO_3. Simultaneously blank was prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 20% Na_2CO_3. A standard curve was prepared using gallic acid. For this, 10 mg gallic acid was dissolved in 100% methanol. Several dilutions of Gallic acid methanol were prepared, *viz.* 2.5, 5, 10, 15 and 25 µg/ml. One ml aliquot of each dilution was taken in a test tube and diluted with 10 ml of distilled water and to this 2.5 ml Folin-Ciocalteu's reagent was added. This was followed by the addition of 2.5 ml of 7.5% $NaHCO_3$ in each test tube. The resulting mixture was left to stand for 30 minutes at room temperature. Absorbance of the standard was measured at 765 nm using UV spectrophotometer against blank. Quantification was done on the basis of a standard curve of gallic acid. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract). Gallic acid equivalent (GAE) T= C x V/M, where GAE is the gallic acid equivalence (mg/ml); V is the volume extract (ml) and M is the weight (g) of the pure plant extract.

The % yield extracts was calculated by using the following formula:

$$\% \text{ yield} = \frac{\text{Weight of extract obtained (g)}}{\text{Weight of plant material (g)}} \text{ X } 100$$

RESULTS AND DISCUSSION

Qualitative estimation of preliminary phytochemicals in *C. vialis*.: The percent yield of *C. vialis* extract has been given in Table 1. The result of qualitative phytochemical analysis is given in Table 2 and 3. Results showed positive results for phenols, tannins, saponins, glycosides, steroids, terpenoids, coumarins in various solvents extracts.

Table 1. Percentage yield of *C. vialis* extracts

Plant part	% yield in various solvent			
	Methanol	Water	Ethyl acetate	Chloroform
Leaves	7.6	3.26	0.5	1.9
Stem	1.89	6.07	NT	NT
Root	4.5	6	NT	NT

NT= Not Tested

Table 2. Phytocompounds in different solvents extracts of leaves of *C. vialis*

Phytocompound	Ethyl acetate extract	Methanol extract	Chloroform extract	Aqueous extract
Protein	-	-	-	-
Phenol	+	+	+	+
Saponin	-	-	+	+
Tannins	-		+	-
Glycosides	+	+	+	+
Steroids	-	+	+	+
Terpenoids	-	-	+	+
Phlobatannins	-	-	-	-
Coumarins	-	+	-	-
Leucoanthocyanins	-	-	-	-

Table 3. Phytocompounds in different solvents extracts of *C. vialis*

Phytocompound	Root		Stem	
	Aqueous extract	Methanol extract	Methanol extract	Aqueous extract
Proteins	+	+	+	+
Phenols	+	+	+	+
Saponin	-	-	-	-
Tannins	-	-	-	-
Glycosides	+	+	+	+
Steroids	+	+	+	+
Terpenoids	-	+	+	+
Phlobatannins	-	-	-	-
Coumarins	-	+	-	+

Phenolic content of the extracts

Different parts of *C. vialis* were extracted with methanol, aqueous, chloroform and ethyl acetate and were subjected for analysis of total phenolic content. The calibration curve showed linearity for gallic acid in the range of 0.25 – 2.5 ìg/ml, with a correlation coefficient (R2) of 0.999 (Fig. 1).

Total phenolic content was estimated by gallic acid (Fig. 2, 3, and 4) and expressed as milligrams of gallic acid equivalent (GAE). Different concentrations, *viz*. 2.5, 5, 7.5, 10 and 15 mg/ml of ethyl acetate, chloroform, methanol and aqueous extracts of leaves were prepared and subjected to quantitative estimations of total phenols. Phenolics in leaves

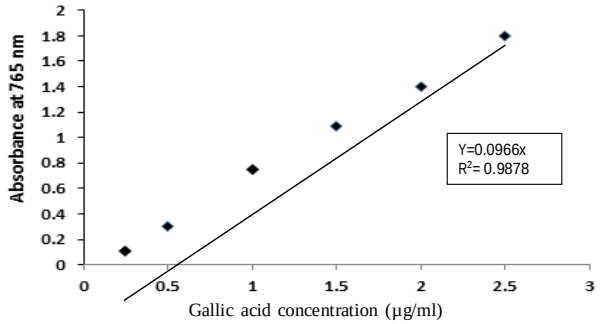

Fig. 1. Calibration plot for phenolic determination

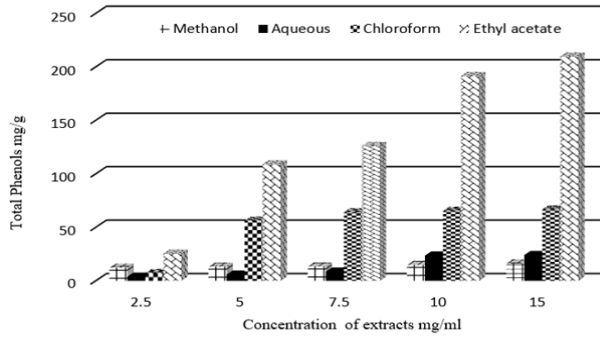

Fig. 2. Total phenols in leaves extracts

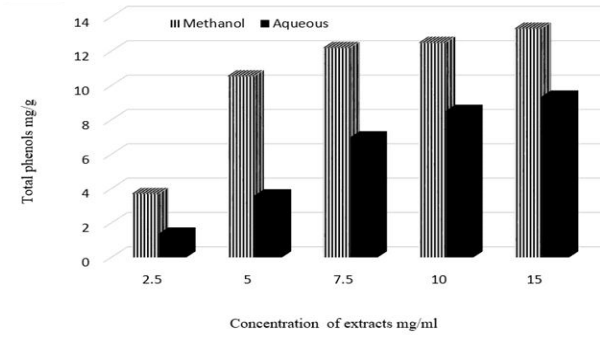

Fig. 3. Total phenols in stem extracts

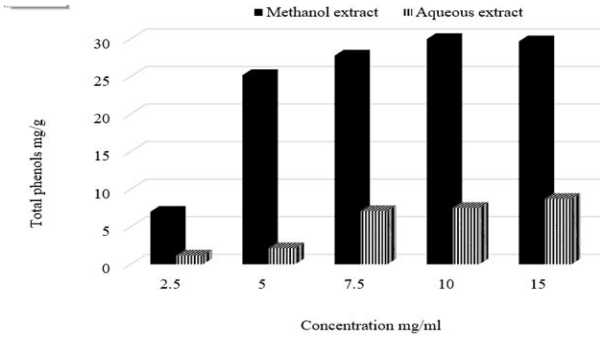

Fig. 4. Total phenols in root extracts

extracted with ethyl acetate were high (209.7 mg/g) followed by chloroform (66.70 mg/g), aqueous (24.06 mg/g) and methanol (16.2 mg/g) at 15 mg/ml concentration. In stem extracts, the phenolics extract in methanol were moderate depending upon the concentration. In root extracts, methanol extract showed high content than aqueous extract.

The presence of phenolics in the root, stem and leave of *C. vialis* indicate its allelopathic effect on its associated flora. Phenolic compounds are known to be responsible for antioxidant, anti-inflammatory, antimicrobial and several other biological properties. In the present study, considerable amount of phenolics were found in *C. vialis*. This has paved way to the agricultural scientists, pharmacologists, biochemists, biotechnologists, cytologists *etc*. to explore potential properties for sustainable use of *C. vialis*, which can be used for the human welfare and which will equally contribute to the protection of loss biodiversity. Further a question rises whether we can consider of designing agro-ecosystems incorporating this weed.

The present work established that *Calyptocrapus vialis* leaf contains most of the phytochemicals. The leaves, root and stem of *C. vialis* contain phenolic compounds, which reveal it's allelopathic nature.

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SUMMARY

Preliminary phytochemical analysis and quantitative estimation of total phenols in root, stem and leaves of *Calyptocarpus vialis* Less. (Asteraceae) were done using different solvents, *viz.* methanol, ethyl acetate, aqueous and chloroform. In all the solvent extracts of leaves, there was presence of phenols, glycosides, steroids, tannins and terpenoids. For quantitative estimations, different concentrations of various solvent extracts were prepared, *viz.* 2.5, 5, 7.5, 10 and 15 mg/ml. Quantitative estimation of total phenols showed that ethyl acetate extract in leaves contained high phenolic contents followed by chloroform, aqueous and methanol extracts. In stem and root, high phenolic content was found in methanol extracts at high concentrations of the plant sample. This seems to be the first report from India.

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