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Research Article

TOTAL PHENOLIC, FLAVONOID, TANNIN CONTENTS, AND ANTIOXIDANT ACTIVITY OF ORGANIC EXTRACTS OF VEGETATIVE AND FLOWERING STAGES OF *MECARDONIA PROCUMBENS* (MILL.) SWALL.

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ABSTRACT

Objective: The objective of the study is to assess the total amount of phenols, flavonoid, and tannin content along with its antioxidant property of two physiological different stages such as vegetative and flowering in *Mecardonia procumbens* (Mill.) Swall.

Methods: subsequent extraction s done by socklet apparatus with solvents of increasing polarity: hexane, acetone, methanol, and to estimated for the biological metabolites.

Results: Stage of the plant significantly affected the quality of the phenols, flavonoids, and tannins. The highest amount of phenol (86.25±1.03 Gallic acid equivalents per gram of dry weight [GAE/g DW]) was reported in acetone extract in flowering sage similar result was with the flavanoid W(14.25±0.52 GAE/g DW). Whereas tannins were high in methanol extract of the flowering stage (49.52±1.02 mg catechin/g DW). The best scavenging activity was found in the flowering stage of acetone extract (3.5±0.06 µg/mL) and total antioxidant property was also in flowering stage of methanol extract (97.48 mg GAE/g DW) the *M. procumbens*.

Conclusion: Extracts of acetone and methanol were more effective and could be used as preservatives in food or pharmaceuticals.

Keywords: Antioxidant property, Flowering stage, *Mecardonia procumbens*, Phenols, Tannins.

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INTRODUCTION

Mecardonia procumbens (Mill.) Swall Synonyms: *Herpenstis pendunculaaris* (Benth.) Small, belongs to the family Plantaginaceae as per the APG system III (earlier Scrophulariaceae) and is identified by one of the authors. It is commonly called baby jump-up in English as garurbramhi in Bengali. The plant is an annual, prostate, and glabrous herb. Stem usually branched, 10–25 cm long, rooting at the lower nodes, four-angled, slightly twisted, leaves opposite, and leaf blade elliptic to ovate. Flowers axillary, 2 per node, subtended by leafy bracts, corolla lemon-yellow, lightly exerted beyond the calyx. Fruit is a capsule, oblong, loculicidally 2-valved, seeds minute.

Medicinally the plant is said to be brain stimulant as well as neurosimulant and the leaves are used treating cuts, wounds, and ringworm.

In plants cellular structures are protected from oxidative effects under stressful conditions by enhanced synthesis of secondary metabolites, these include vitamins, terpenoids, carotenoids, essential oils, and phenolic compounds [1]. The distribution of secondary metabolites may change during plant development are related to the climatic conditions of the plants habitat which stimulate the biosynthesis of secondary metabolites such as polyphenols, flavonoids, tannins. The content of the secondary metabolite depends on the intrinsic (genetic and extracting solvent) and extrinsic (environment and development stage) factors [2]. Antioxidant activity is due to their redox properties which allow them to act as reducing agent or hydrogen atom donors, which act as natural antioxidants function as free radical scavengers and chain breakers, complexes of pro-oxidant metal ions, and quenchers of singlet oxygen formation [3].

MATERIALS AND METHODS

Preparation of plant extract

The plant material is collected from Govindram Seksaria Science College college campus, Belagavi, Kanataka, India. The plants were washed in running tap water to remove adhered particles then air-dried at room temperature and made into a fine powder using kitchen blender and stored in airtight container till the further use. The extraction is done using socklet apparatus with solvents of increasing polarity: hexane, acetone, methanol, and water using appropriate temperature depending on the solvent. The extracts were evaporated to dryness and stored until analysis.

Total phenol content

Total phenol content is analyzed using method described by Dewanto *et al.* [4]. Briefly, an aliquot of diluted extract was added to 0.5 mL of distilled water and 1.25 mL of 7% Na_2CO_3. The solution was then adjusted with distilled water to the final volume of 3 mL and mixed. The reaction mixture is kept 15 min in dark for incubation at room temperature 24±2°C. The absorbance is taken at 760 nm. The total phenol content of the plant extract is expressed as mili gram of Gallic acid equivalents per gram of dry weight (mg GAE/g DW) from the calibration curve with Gallic acid. Al samples were analyzed in three replicates.

Total flavanoid content

Total flavanoid content was measured by colorimetric assay as described by Dewanto *et al.* [4]. Briefly, an aliquot of diluted sample of plant extract is mixed with standard Follin catechin solution to which 75 mL of $NaNO_2$ (5%w/v) added and mixed for 6 min before the addition of 0.15 mL $AlCl_3$ (10% w/v). After incubation for 5 mi 0.5 mL of NaOH (0.1N v/v) were added. The final volume was adjusted to 2.5 mL with distilled water and mixed thoroughly. Absorbance was

determined at 510 nm against a blank. The total flavonoid content is expressed as milligram of caechin erg ran of dry weight (mg CE/g DW) against the calibration curve of catechin. All samples were analyzed in triplicate.

Total condensed tannins

Total condensed tannins were determined according to the method described by Sun [5]. To 50µL of diluted sample 3L of 4%, vanillin solution in methanol and 1.5 mL of concentrated HCl were added. The mixer was allowed to stand for 15 min at room temperature 28±2°C and absorbance were measured at 500 nm against methanol as the blank. The amount of total condensed tannins I expressed as mg catechin/g DW. All samples were analyzed in triplicate.

1,1-diphenyl-2-picrylhydrazyl (DPPH) assay

The DPPH assay is determined by the electron-donating ability of the obtained extracts and was measured by bleaching a purple solution of DPPH radical were described by the method of Hanato *et al* [6]. Extracts (0.1, 5, 10, 50 and 100 mL) were added to 0.5 mL of 0.2 mmol/L DPPH-methanol solution. The reaction mixture is kept at room temperature and the absorbance was measured against the blank at 517 nm. The percentage of inhibition of free radicals was calculated from the formula;

$$\% \text{ of inhibition} = (A_{blank} - A_{sample}/A_{blank}) \times 100$$

Where A_{blnak} is absorbance of the blank and A_{sample} is the absorbance of sample with plant extract. The concentration of the extract used of the extract caused 50% inhibition (IC_{50}) was calculated from the regression equation for the concentration of extract and percentage inhibition. Butylated hydroxyl toluene was used as a positive control. All samples were analyzed in triplicate.

Total antioxidant capacity

Total antioxidant assay is based on the method described by Prieto *et al.* [7] the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate Mo(V) complex at acidic pH. Briefly an aliquot (0.1 mL) of pant extract was added to 1 mL of reagent solution (0.6 mol/L H_2SO_4, 28mmol/L Na_3PO_4 and 4.0 mmol/L ammonium moybdate). The reaction mixture is incubated in the thermal block at 95°C for 80 min and cooled at room temperature. The absorbance of the reaction mixture is was measured at 695 nm against the blank. Antioxidant capacity was measured as mg mg GAE/g DW. The calibration curve range was 0–500 µg/mL. All samples were analyzed in triplicate.

Statistical analysis

Data were analyzed using IBM SPSS (Version 20) statistical software. Analysis of variance and Duncan Multiple Range Test was used to compare any significant difference between solvents and samples. The values were expressed as mean±SD at 5% level of probability.

RESULTS

Total phenols, flavanois and tannins

Table 1 shows the results of *M. procumbens* plant extracts, total phenol content was higher in flowering stage (86.25) and is almost double of methanolic extract and almost eight times higher to the water extract. Phenols in hexane were least amount (0.85). In the vegetative stage acetone extract (45.20) was found to have higher phenolic content followed by methanol, water, and hexane. Similar tendency was observed in flavanoid where in flowering stage (14.25) and is almost thrice as of methanolic extract (5.02). In water extract flavonoids are found to be very less followed by hexane was (0.09). In the vegetative stage methanolic extract (8.62) was found to have higher flavonoid content followed by acetone, water, and hexane. Meanwhile, tannins were high in methanolic extract both in flowering (49.52) and vegetative (18.23) stage followed by acetone, water, and hexane, respectively.

DPPH radical-scavenging activity

Extracts of *M. procumbens* plant in flowering stage showed the higher antioxidant activity than the vegitative stage (Table 2). Acetone extract in flowering stage (3.5) has the higher antioxidant capacity followed vegitative stage (4.6) then methanol and most popuar polar solvent water has moderate activity while hexane has the least anti-radical activity which is true for both vegetative and flowering stage, respectively. Thus phenolic compounds from acetone and methanol extracts of *M. procumbens* were more efficient antioxidants than Butylated hydroxytoluene (12.96).

Total antioxidant activity

The total antioxidant capacity of *M. procumbens* was higher in plants collected at flowering stage than that of vegetative stage (Table 3) and it is varied almost double in each extracted solvent in vegitative and flowering stage. The methanol (97.48) extract of the plant in flowering stage has highest amount of total antioxidant activity followed by acetone, water, and hexane (31.21). Similarity was found in the vegetative stage where methanol (48.52) extract has highest amount of total antioxidant activity followed closely by acetone, hexane, and water.

DISCUSSION

In the present study of *M. procumbens* the phenol content depended on the solvent used and its polarity. Total phenols were high in acetone extract at flowering stage than the vegetative stage. Flavanoids were reported high in acetone in flowering stage whereas in vegetative stage

Table 1: Total phenol (expressed in GAE/g DW) flavonoid and tannin (expressed in mg catechin/g DW) content in *M. procumbens* vegetative and flowering stage

Solvent	Phenols		Flavanoid		Tannins	
	Vegetative	Flowering	Vegetative	Flowering	Vegetative	Flowering
Hexane	0.96±0.02c	0.85±0.05c	0.86±0.02c	0.09±0.01c	0.5±0.01c	2.36±0.05d
Acetone	45.20±0.96a	86.25±1.03a	5.63±0.9b	14.25±0.52a	7.52±1.02b	32.52±0.14b
Methanol	39.25±0.25a	46.24±3.05b	8.62±1.02a	5.02±0.30b	18.23±1.05a	49.52±1.02a
Water	10.25±0.17b	11.25±0.96bc	1.02±0.05bc	2.52±0.14b	3.58±0.08b	8.25±1.08c

Values (means of three replicates) followed by different ettres are significantly different p<0.05. *M. procumbens: Mecardonia procumbens*, GAE/g DW: Gallic acid equivalents per gram of dry weight

Table 2: Scavenging activity exrpressed as median inhibitory concentration (µg/mL) in *M. procumbens* vegetative and flowering stage

Stage	Hexane	Acetone	Methanol	Water	Butyated hydroxytoluene
Vegitative	>1000a	4.6±1.09c	5.29±0.61c	59.63±2.09b	12.96±0.05bc
Flowering	>1000a	3.5±0.06c	5.50±0.02c	36.25±0.85b	

Values (means of three replicates) followed by different ettres are significantly different p<0.05. *M. procumbens: Mecardonia procumbens*

Table 3: Total antioxidant capacity (expressed as mg GAE/g DW) in *M. procumbens* vegetative and flowering stage

Solvent	Vegetative	Flowering
Hexane	12.03[b]	31.21[b]
Acetone	45.21[a]	84.21[a]
Methanol	48.52[a]	97.48[a]
Water	11.09[b]	66.21[c]

Values (mean of three replicated) followed by different letters are significantly different at p<0.05. *M. procumbens: Mecardonia procumbens*, GAE/g DW: Gallic acid equivalents per gram of dry weight

methanol content has the highest amount. Similar results were seen in *Petroselinum crispum* [8] and *Fumaria vaillantii* [9]. However, tannins were found to be high in methanolic extract for both vegetative and flowering stages.

It is found that the recovery of polyphenols from the plant material is influenced by their solubility in the extraction solvent, type of solvent, and also the degree of polymerization of phenols along with other constituents of plant material with the formation of insoluble complexes [10]. Earlier reports reveal that solvent polarity plays important role in the phenolic solubility [11]. Physiological stage can also affect composition of and content of biological active compounds such as in the present study which is true with the studies done on *Limonium delicatulum* [12] and *Origanum majorana* [13]. It is reported that the phenol content and the antioxidant capacity are strongly affected by the growing season in cultivated plants in Japan [14]. and some of the vegetables such as eggplant [15].

The phenol contains a wide variety of antioxidants and is difficult to measure each compounds individually, most of the methods it is measured by the scavenging radicals [12]. In the present study of *M. procumbens* highest scavenging activity was found in acetone extract for both vegetative as well as in flowering stage whereas total antioxidant was high in methanol extract for both the vegetative and flowering stage. The variations in the phenolic compounds are clearly observed in the species like Plantago [16] and *Silybum marianum* [17] where flowering stage has the high range of polyphenols. Structurally, phenols contain aromatic ring with one or more hydroxyl group which donates hydrogen atoms or electrons to scavenge free radicals or to chelate [2] which give to the color to the reaction mixture.

Ethanolic extract has the greater affinity for inducing antioxidant activity which is also reported by different author in different plant which is similar to the present study such as *Cichorium spinosum* [18] *Celosia argentea* [19].

CONCLUSION

Extracts in acetone and methanol were more effective than those in hexane and water. In addition, the plant in flowering stage had the greater activity than in the vegetative stage. These results indicate that selective extraction of bioactive molecules from natural sources such as holotype species with appropriate solvent can provide fractions with biological activity that could be used as preservatives in food or pharmaceuticals.

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AUTHOR'S CONTRIBUTION

The plant collection, extraction were done by CN and AMG. Experimental work, data analysis, and manuscript corrected by AMG. Both the authors read and approved the final manuscript.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest to publish the article.

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