INTRODUCTION

Mecardonia procumbens (Mill.) Swall Synonyms: Herpenstis penduncularis (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 four-angled, slightly twisted, leaves opposite, and leaf blade elliptic to ovate. Fruit is a capsule, oblong, loculicidally 2-valved, seeds minute.

Medicinally the plant is said to be brain stimulant as well as nervinstimulant and the leaves are used as 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 campus, Belagavi, Karnataka, 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 socket 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 [8.62±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 flavonoid W[1.425±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.
determined at 510 nm against a blank. The total flavonoid content is expressed as milligram of catechin equivalent 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 50 µL of diluted sample 3 mL of 4%, vanillin solution in methanol and 1.5 mL of concentrated HCl were added. The mixture was allowed to stand for 15 min at room temperature 28±2°C and absorbance was measured at 500 nm against methanol as the blank. The amount of total condensed tannins 1 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 Ilanato 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:

\[
%\text{ of inhibition} = \left(\frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{sample}}}\right) \times 100
\]

Where A_{\text{sample}} is absorbance of the blank and A_{\text{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 hydroxytoluene 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$_2$SO$_4$, 28mmol/L Na$_2$PO$_4$ and 4.0 mmol/L ammonium molybdate). 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 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, flavanoids 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 flavonoid 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 (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 vegetative stage (Table 2). Acetone extract in flowering stage (3.5) has the higher antioxidant capacity followed vegetative stage (4.6) then methanol and most popular polar solvent water has moderate activity while hexane has the least antioxidant 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 the 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 vegetative 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 (49.52) 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 | Flavonoids | 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±0.05c |
| Acetone | 45.20±0.96a | 86.25±1.03a | 5.63±0.9b | 14.52±0.52a | 7.5±1.02b | 32.52±0.14b |
| Methanol| 39.25±0.25a | 46.24±3.05b | 8.62±1.02a | 5.02±1.08c | 18.23±1.05a | 49.52±1.02a |
| Water   | 10.25±0.17b | 11.25±0.96c | 1.02±0.05bc | 2.52±1.4b | 3.59±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 expressed as median inhibitory concentration (µg/mL) in M. procumbens vegetative and flowering stage

| Stage  | Hexane | Acetone | Methanol | Water | Butyated hydroxytoluene |
|--------|--------|---------|----------|-------|-------------------------|
| Vegetative | >1000a | 4.6±1.09c | 5.29±0.61c | 59.6±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 a    | 31.21 b   |
| Acetone  | 45.21 a    | 84.21 b   |
| Methanol | 48.52 b    | 97.48 c   |
| 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 delicatum* [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.

Ethanol 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 factions with biological activity that could be used as preservatives in food or pharmaceuticals.

ACKNOWLEDGMENT

The authors acknowledge the respective departments for extending facilities to conduct the present work.

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.

AUTHOR’S FUNDING

The present work is completely self-funded.

REFERENCES

1. Mazid M, Khan TA, Mohammad F. Role of secondary metabolites in defense mechanisms of plants. Biol Med 2011;3:232-49.
2. Friatianni F, Tucci M, De-Palm M, Pepe R, Nazzaro F. Polyphenolic composition in different parts of some cultivars of globe artichoke (*Cynara cardunculus* L. var. *Scolymus* (L.) Fiori). Food Chem 2007;104:1282-6.
3. Tosun M, Celik F, Ercidi SO, Yilmaz S. Bioactive contents of commercial cultivars and local genotypes of walnut (*Juglans regia* L.). In: International Conference on Environmental and Agriculture Engineering. Vol. 15. Bangkoč Kasetart University; 2011.
4. Dewanto X, Wu K, Adom K, Liu RH. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J Agric Food Chem 2005;53:3010-4.
5. Sun B, Richardo-Da-Silvia JM, Spranger I. Critical factors of vanillin assay for catechins and proantho cyanidins. J Agric Food Chem 1998;46:4267-74.
6. Hatanomo T, Kagawa H, Yasuhara T, Okada T. Two new flavonoids and other constituents in licorice root their relative astringency and radical scavenging effect. Chem Pharm Bull 1988;36:2090-7.
7. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosho-molybdenum complex: Specific application to the determination of Vitamin E. Anal Biochem 1999;269:337-41.
8. Aisha M, Ahmed A, Fatma M, Abdelkhalak ME, Kalid AK. Morpholgicca and chemical characters of *Petroselinum crispum* (Mill) subjected to some biostimulants. Asian J Plant Sci 2018;17:96-106.
9. Mohammad M, Seyd M, Kialeghi M, Leila M. Total phenolic content and antioxidant activity of *Fumaria vaillantii* extract at three phenological stages assessed by various methods. Intern J Hort Sci Tech 2018;5:93-102.
10. Galvaz CJ, Martin-Cordero P, Houghton AM. Antioxidant activity of methanol extracts obtained from *Plantago* species. J Agric Food Chem 2005;53:1927-33.
11. Naczk M, Shahidi F. Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. J Pharm Biomed Anal 2006;41:1523-42.
12. Medini F, Fellah H, Ksouri R, Chedly A. Total phenolic, flavonoid and tannin contents and antioxidant and antimicrobial activities of organic extracts of shoots of the plant *Limonium delicatum*. J Taibah Uni Sci 2014;8:216-24.
13. Khadhri A, Bouali I, Aouadhi C, Labgel MC, Masson E, Pizzi A. Determination of phenolic compounds by MALDI-TOF and essential oil composition by GC-MS during three development stages of *Origanum majorana* L. Biomed Chromatogr 2019;33:e4665.
14. Ichih MK, Shiro M, Keijiro N, Yumico T, Tetsuo S, et al. Antioxidant capacity and polyphenols contents of extracts from crops cultivated in Japan and the effect of the cultivation. Environ Food Sci Technol Res 2013;19:69-79.
15. Naser JK, Layth SA, Ahmed AR, Joseph C. Chemical composition and antioxidant capacity of eggplant parts during vegetative and flowering stage. J Phys 2021;1294:092013.
16. Lacramioara O, Mihaela I, Marius NG, Maria MZ. Antioxidant activity of *Plantago* species in vegetative and flowering stage. Iran J Public Health 2015;4:142-4.
17. Sulas L, Re GA, Bollita S. Chemical and productive properties of two Sarinian milk thistle (*Silybum marianum* (L.) Gaertn) population as sources of nutrients and antioxidants. Genet Resour Crop Evol 2016;63:315-26.
18. Spyridon A, Petropoulos A, Ángela F, Antoniadias V, Georgia N, Lilian B, et al. Chemical composition and antioxidant activity of *Cichorium spinosum* L. leaves in relation to developmental stage. Food Chem 2018;239:946-52.
19. Adegbaju OD, Otunola GA, Afolayan AJ. Effect of growth stage and season on the phytochemical content and antioxidant activities of crude extract of *Celosia argentea* L. Heliyon 2020;6:e04086.