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Preliminary Phytochemical Analysis and Antioxidants Activities of Ethanolic Extract from *Gomphrena serrata* Whole Plant

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Abstract

The present study was designed to investigate the phytochemical analysis and antioxidant activities of the whole plant of *Gomphrena serrata*. *Gomphrena serrata* widely distributed in South America, North America, and India. The parts of these plants are used as traditional medicine for the treatment of several ailments. This study aims to assess the phytochemical and free radical scavenging of ethanolic extract of *G. serrata* present in the whole plant. The preliminary phytochemical study was performed by standard method. The whole plant of *G. serrata* proved the presence of bioactive constituents such as carbohydrates, alkaloids, steroids, glycosides, triterpenoids, protein and amino acids, saponins, as well as flavonoids. The *in vitro* antioxidant study was performed on the ethanolic extract of shade-dried of the whole plant, which determined by hydrogen peroxide, hydroxyl radical, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) 100 µg/ml assay and was compared to ascorbic acid as the positive control. The ethanolic extract of the whole plant of *G. serrata* shows the strong free radical scavenging activity. The present study was the proof for ethanol extract of *G. serrata* which have medicinally significant and bioactive compounds since these plant species are used as traditional medicine for the treatment of various diseases.

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INTRODUCTION

The traditional knowledge forms of codified systems of medicine are exists in the forms of Ayurveda, Unani and Siddha (Sen & Chakraborty, 2016). India is the oldest, richest diverse cultural traditions associated with the use of medicinal plants. This knowledge is accessible from thousands of medical test and manuscript (Kumar *et al*., 2017). Since ancient time, mankind was using herbal plants for treatment of certain diseases. The study of traditional medicine based on bioactive compounds in the plants is called as ethnomedicine study (Adhikari *et al*., 2019).

Among medicinal plants, the substances having medicinal value have been extensively used for treating various disease conditions (Sofowora *et al*., 2013). Herbs being easily available to human beings have been explored to the maximum for their medicinal properties (Ekor, 2013). Phytoconstituents are the natural bioactive compounds found in plants. This phytoconstituents work with nutrients and fibers to form an integrated part of defense system against various forms of diseases and stress conditions (Altemimi *et al*., 2017).

Gomphrena serrata, the plants belong to the family of amaranthaceae are very rich source of bioactive constituents like carbohydrate, alkaloids, steroids, glycosides, and triterpenoids. In general, the family of amaranthaceae contains nearly 60-70 exotic species (Nandini *et al*., 2018). The genus Gomphrena, contain

about 138 species, some of the important species include *G. boliviana, G. celosioides, G. globose, G. haenkeana, G. macrocephala, G. martiana, G. meyeniana, G. perennis*, and *G. pulchella*. The various parts of this plant are used in India for treatment of various ailments need for the traditional healers, including treatment of asthma, diarrhea, indigestion, dermatitis, hay fever, and others (Rahman & Gulshana, 2014).

Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are products of normal cellular metabolism. These free radicals are the fundamental to any biochemical process and represent as an essential part of aerobic life and metabolism (Di Meo *et al*., 2016). Antioxidant are the molecules which have capability to prevent the oxidation of other molecules (Kurutas, 2016). The objective of the study was to analyses phytochemical constituent and antioxidant potential of *G. serrata*. The plant extracts determined by hydrogen peroxides, hydroxyl radical and DPPH assays which are designed by the EC50 and compared with the standard ascorbic acid. However, there are no reports on phytochemical analysis of whole plant of ethanol extract of *G. serrata* have been reported.

MATERIALS AND METHODS

Plant collection

The fresh whole plants of *G. serrata* were collected from the local area of Bharathinagara, Mandya, Karnataka. The plants were identified and authenticated by Botanist Dr. Gurukar Mathews, Head of the Department Bharathi College of Post-Graduation and Research Centre, Bharathinagara, Maddur, Mandya, Karnataka, India.

Extraction

After the collection of whole plant of the *G. serrata* was wash thoroughly with running tap water, cut in to small pieces, and shade dried. The dried whole plant then pulverized separately into coarse powder by a mechanical grinder. As much as 100 g of powdered *G. serrata* was carried out by hot extraction process using Soxhlet apparatus with ethanol as solvent for 72 hours at 50°C. The distillates were collected and distilled separately to yield the extracts. These extracts concentrated using vacuum rotary evaporator to obtain crude extract. It turned into a greenish black color with yield of 10.8%. The extract was kept in a desiccator over anhydrous calcium chloride until used.

Phytochemical screening

Small quantity of freshly prepared extract of *G. serrata* were subjected to quantitative chemical tests for identification of various phytoconstituents. Phytochemical investigations were carried out as per the standard methods set by WHO (Khandelwal, 2006; Khadabadi *et al*., 2013).

Carbohydrates test

1. Molisch test

   As much as 1 ml of extract was treated with the compounds of β-naphthol and added with concentrated sulphuric acid along the sides of the test tube. Purple or reddish violet color was formed at the junction between two liquids, which indicated the presence of carbohydrates.

Alkaloids test

1. Dragendorff test

   As much as 1 ml of extract was treated with 1 ml of Dragendorff reagent. Orange red precipitate was formed which indicates the presence of alkaloids.
2. Wagner test

   As much as 1 ml of extract was treated with 1 ml of Wagner's reagent. Reddish brown precipitate was formed, which indicates the presence of alkaloids.
3. Mayer test

   As much as 1 ml of extract was treated with 1-2 drops of Mayer's reagent. Cream colored precipitate was formed, which indicates the presence of alkaloids.

4. Hager test

   As much as 1 ml of extract was treated with 3 ml of Hager's reagent. Yellow precipitate was formed, which indicates the presence of alkaloids.

Glycosides test

1. Keller-Killiani test

   As much as 2 ml of extract was dissolved in acetic acid containing trace of ferric chloride and transferred to the surface of concentrated sulphuric acid. At the junction of two liquids reddish brown color was formed, which gradually blue color due to the presence of glycosides.
2. Borntrager test

   As much as 1 ml of diluted H_2SO_4 was added with 2 ml of extract. The mixture was boiled, filtered, and extracted with ether or chloroform. Organic layer was separated to which ammonia was added. Pink, red, or violet color was produced in organic layer, which indicated the presence of glycosides.

Phytosterols and triterpenes test

1. Liebermann–Burchard test

   As much as 1 ml of extract was treated with 2 ml of chloroform in a dry test tube. Then 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid were added. The solution was turned into red, then blue, and finally green in color, which indicates the presence of phytosterols.
2. Salkowski test

   As much as 1 ml of extract was treated with 1 ml of chloroform and added 2 ml of concentrated H_2SO_4. Bluish red and purple color was formed in chloroform layer, which indicate the presence of triterpenes.

Tannins and flavonoids test

1. Gelatin test

   As much as 1 ml of extract was treated with 1% gelatin solution containing sodium chloride. Formation of white precipitate indicates the presence of tannins.
2. Lead-acetate test

   As much as 1 ml of extract was treated with 10% lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.
3. Shinoda test

   As much as 1 ml of extract was treated with a few fragments of magnesium and concentrated HCl were added. Appearance of magenta color after few minutes indicates presence of flavonoids.

Proteins and amino acids test

1. Biuret test

   As much as 1 ml of extract was treated with 1 ml of 40% NaOH and 2 drops of 1% copper sulphate. Appearance of violet color indicates the presence of proteins.
2. Xanthoproteic test

   As much as 1 ml of extract was treated with 1 ml of 20% of sodium hydroxide or ammonia. Appearance of orange color indicates the presence of aromatic amino acid.

Fixed oils and fats test

1. Spot test

   As much as 1 ml of extract was applied as a spot in filter paper. Appearance of a clear-transparent spot indicates the presence of fixed oils.

Saponins test

1. Foam test

   As much as 1 ml of extract was treated in hot water sufficiently, and after cooled until room temperature then shake vigorously for 10 seconds. It was produced the foam then 1% HCl was added. Foam that lasts for not less than 10 minutes indicates the presence of saponins.

In vitro antioxidant test

Each sample was dissolved in distilled methanol to make a concentration of 20-100 µg/ml and then diluted to prepare the series concentrations for antioxidant assays. Reference Ascorbic acid was used for standard comparison in all assays.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of *G. serrata* was measured according to a method described previously with some modification (Smirnoff & Cumbes, 1989). Briefly, the different concentration of ethanol extract of *G. serrata* was mixed with 1 ml of 9 mM of Salicylic acid, 1 ml of 9 mM of Ferrous sulphate, and 1 ml of 9 mM Hydrogen peroxide, respectively. The mixture was then incubated at 37°C for 60 min in a water bath. After incubation period, the absorbance of the mixtures was measured at 510 nm. The activity of hydroxyl radical scavenging (%) was calculated as follows:

$$\% \text{ inhibition} = \frac{\text{absorbance of control - absorbance of sample}}{\text{absorbance of control}} \times 100$$

Hydroxyl peroxide scavenging activity

Hydrogen peroxide solution (4 mM) was prepared in 50 mM phosphate buffer pH 7.4. As much as 0.1 ml of aliquots from different concentration sample solution was transferred into the test tubes and their volumes were made up to 0.4 ml with 50 mM phosphate buffer. After addition of 0.6 ml hydrogen peroxide solution, mixed solution and absorbance of the hydrogen peroxide at 230 nm was determined after 10 minutes, against a blank (Ruch *et al*., 1989). The abilities to scavenge the hydrogen peroxide was calculated using the following equation:

$$\% \text{ inhibition} = \frac{\text{absorbance of control - absorbance of sample}}{\text{absorbance of control}} \times 100$$

DPPH free radical scavenging activity

As much as 2.36 g of the DPPH was dissolved in 100 ml of methanol to get 6 x 10^{-5} M methanolic solution of DPPH. A series concentration of standard ascorbic acid and *G. serrata* extract that is 20, 40, 60, 80, and 100 µg/ml were prepared by diluting with methanol (Pavithra & Mani, 2019). As much as 1 ml of each diluted standard and test solution were mixed with 3 ml of DPPH solution in each test tube. Control solution was prepared by adding 1 ml of methanol and 3 ml of DPPH. The test tubes were covered with aluminum foil to protect from light and kept in dark place for 15 minutes. Methanol was used as blank. Absorbance of standard, control, and test extract was measured at 517 nm using UV-Visible spectrophotometer. The % inhibition was calculated by using following formula and compared with the values of standard ascorbic acid:

$$\% \text{ inhibition} = \frac{\text{absorbance of control - absorbance of sample}}{\text{absorbance of control}} \times 100$$

Statistical analysis

All the experiment was carried out in triplicate and data reported are mean ± standard deviation. Then EC_{50} was calculated from the graph obtained by percentage of inhibition was plotted against concentration.

RESULTS AND DISCUSSION

The *G. serrata* extract was subjected for qualitative chemical analysis for the identification of various phytoconstituents, revealed the presence of carbohydrates, alkaloids, glycosides, phytosterols and triterpenes, tannins and flavonoids, proteins and amino acids, fixed oils and fats, and saponins. Since all these compounds were found to be present in the extracts, it might be responsible for the potent antioxidant capacity of *G. serrata*. The preliminary phytochemical screenings are helpful in finding phytoconstituents in the plant material that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compound (Shrestha *et al*., 2015). Detail

results of each phytochemical screening tests was presented in the **Table I**.

Table I. Preliminary phytochemical analysis of ethanolic extract of *G. serrata*

Phytochemical	Test	Result	Presence
Carbohydrates	Molisch	Reddish-violet	+
Alkaloids	Dragendorff	Orange precipitate	+
	Wagner	Brown precipitate	+
	Mayer	Cream precipitate	+
	Hager	Yellow precipitate	+
Glycosides	Keller-Killiani	Bluish brown	+
	Borntrager	Reddish-violet	+
Phytosterols and triterpenes	Liebermann–Burchard	Greenish blue	+
	Salkowski	Bluish red	+
Tannins and flavonoids	Gelatin	White precipitate	+
	Lead-acetate	Yellow precipitate	+
	Shinoda	Magenta	+
Proteins and amino acids	Biuret	Violet	+
	Xanthoproteic	Orange	+
Fixed oils and fats	Spot	White precipitate	+
Saponins	Foam	Foaming over 10 seconds	+

In vitro antioxidant test

Hydroxyl radical scavenging activity

The scavenging activity of ethanol extract of *G. serrata* on hydrogen peroxide scavenging activity is presented in **Table II**. The percentage inhibitions were increased with increasing concentrations of the extracts as presented in **Figure 1**. Free radicals are known to play very important role in a wide variety of pathological manifestations. Antioxidants fight against free radicals and protect us from various ailments. They exert their action either by scavenging the ROS or protecting the antioxidant defense mechanisms (Umamaheswari & Chatterjee, 2007). In biological systems, hydroxyl radical are the most powerful radicals evolved from hydrogen peroxide and superoxide anions in metal ions presence. Hydroxyl radical can damage any cells in the body and responsible for many pathological conditions in DNA, lipids, as well as proteins and can cause mutagenesis, cancer, and cytotoxicity (Phaniendra *et al.*, 2015).

Table II. Hydroxyl radical scavenging activity of *G. serrata*

Concentration (µg/ml)	% inhibition ± SD	
	Ethanolic extract of *G. serrata*	Ascorbic acid
20	48.13 ± 0.13	50.67 ± 1.07
40	54.89 ± 0.28	61.65 ± 0.20
60	62.74 ± 0.26	72.85 ± 0.77
80	79.45 ± 0.26	81.98 ± 0.29
100	87.56 ± 0.30	92.46 ± 0.28
EC_{50} (µg/ml)	**27.98**	**17.8**

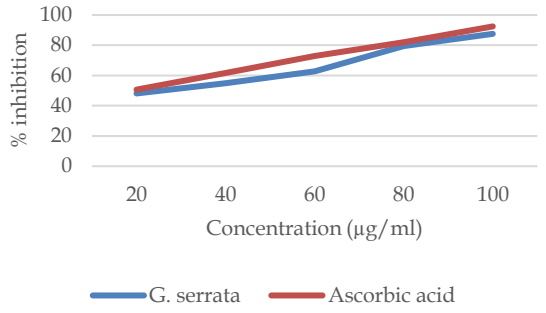

Figure 1. Hydroxyl radical scavenging activity of *G. serrata*

Hydroxyl peroxide scavenging activity

The hydrogen peroxide scavenging activity of ethanol extract of *G. serrata* is presented in **Table III**. The plant extract exhibited antioxidant activity at all the concentration of test solutions, with the increase in concentration of the plant extract also increasing the percentage of antioxidant activity. Among all concentration, maximum antioxidant activity was observed at 100 µl/ml as presented in **Figure 2**. Hydrogen peroxide occurs naturally at low concentration levels in the air, water, human body, plants, microorganisms and food (Gülçin *et al.*, 2005). Hydrogen peroxide is quickly break down into water and oxygen. This will produce hydroxyl radicals (•OH) that can initiate lipid peroxidation and cause DNA damage. Ethanolic extract of *G. serrata* efficiently scavenging hydrogen peroxide which may be attributed

to the presence of phenolic groups that could donate electrons to hydrogen peroxide, thereby neutralizing it into water (Pizzino *et al*., 2017).

Table III. Hydroxyl peroxide scavenging activity of *G. serrata*

Concentration (µg/ml)	% inhibition ± SD	
	Ethanolic extract of *G. serrata*	Ascorbic acid
20	42.53 ± 0.15	47.61 ± 0.30
40	53.72 ± 0.23	56.36 ± 0.11
60	65.48 ± 0.38	68.33 ± 0.14
80	72.84 ± 0.43	76.31 ± 0.41
100	81.75 ± 0.42	85.15 ± 0.27
EC_{50} (µg/ml)	**32.8**	**24.74**

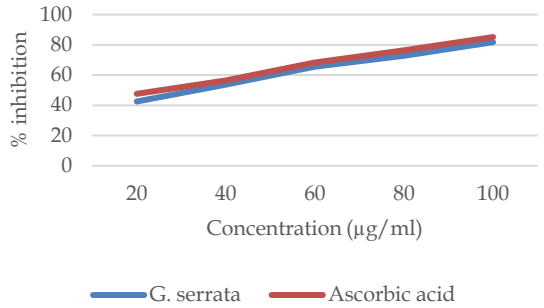

Figure 2. Hydroxyl peroxide scavenging activity of *G. serrata*

DPPH free radical scavenging activity

The DPPH free radical scavenging activity of ethanol extract of *G. serrata* is presented in **Table IV**. The stable radical DPPH had been used widely for the determination of primary antioxidant activity. The DPPH antioxidant assay is based on the ability of a stable free radical to decolorize in the presence of antioxidants (Kedare & Singh, 2011). Among successive solvent of extracts, the highest percentage inhibition by DPPH radical scavenging assay exhibited in ethanolic extract 81.66 ± 0.11 at 100 µg/ml and the lowest percentage inhibition by DPPH radical scavenging assay exhibited in ethanolic extract 42.67 ± 0.15 at 20 µg/ml. The mean EC_{50} value of extract is 33.23 µg/ml and for standard ascorbic acid is 25.43 µg/ml. The percentage inhibitions were increased with increasing concentrations of the extracts as presented in **Figure 3**. That showed the scavenging effect on the DPPH radical increase sharply with increasing concentration of the sample and standards.

Table IV. DPPH free radical scavenging activity of *G. serrata*

Concentration (µg/ml)	% inhibition ± SD	
	Ethanolic extract of *G. serrata*	Ascorbic acid
20	42.67 ± 0.15	47.73 ± 0.48
40	52.73 ± 0.09	56.37 ± 0.46
60	65.72 ± 1.05	68.11 ± 0.32
80	72.85 ± 0.31	76.32 ± 0.31
100	81.66 ± 0.11	87.15 ± 0.38
EC_{50} (µg/ml)	**33.23**	**25.43**

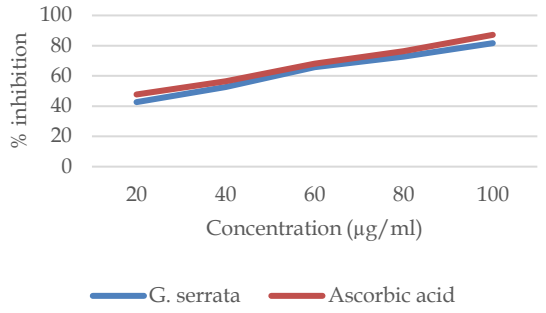

Figure 3. DPPH free radical scavenging activity of *G. serrata*

CONCLUSION

The phytochemical screening showed that the whole plant of *G. serrata* extract contain a mixture of phytochemicals as carbohydrates, alkaloids, glycosides, phytosterols and triterpenes, tannins and flavonoids, proteins, fixed oils, as well as saponins. It is also known that whole plant extract of *G. serrata* also exhibit good scavenging effects on hydroxyl radical, hydrogen peroxide, and DPPH method. In conclusion, the high antioxidant activity exhibited by *G. serrata* extract provided justification for the therapeutic use of this plant in folkloric medicine. Further research is needed for *G. serrata* to identify compounds that have pharmacological properties using an appropriate assay model.

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