



Phytochemical Screening and Antibacterial effects of *Gomphrena globosa* L. Flower Extracts

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ABSTRACT

Medicinal plants are the only green alternative to manmade drugs and gaining a lot of attention. The potential pharmacological actions are attributed to the presence of phytochemical constituents. Natural products from plants have always been a source for the treatment of many human diseases. Traditionally, people have been using these plant sources to treat many disorders and diseases. One such plant is *Gomphrena globosa* L. which has an abundance of phytochemicals and medicinal properties., commonly called Globe amaranth belongs to the family Amaranthaceae. It has been in practice of traditional medicine systems years ago. This study was focused to screen the active phytochemicals and to identify the antimicrobial activity of *Gomphrena globosa* L. flower extract.

KEY WORDS: Phytochemicals, Globe Amaranth, Flower Extracts

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1. INTRODUCTION

Plants are the main natural source of numerous phytochemicals, although only a certain amount have been isolated and identified. Nutritional epidemiology has investigated the relation between diet and human health, reporting positive evidence on the role of phytochemicals (Aguilera, 2021). The interest of the scientific class in the study of compounds of plant origin is increasing worldwide, especially in developing countries where the use of herbal medicines is widely used for their basic health needs (Aguilera, 2018).

Natural bioactive compounds from plants perform specific biological activities and modify different physiological functions to improve health of human being (Niaz et al., 2020). The studies carried out to date affirm that these compounds can reduce the incidence of several chronic diseases, including cardiovascular, obesity, diabetes, and cancer diseases, as well as high blood pressure and inflammation. Vegetables, fruits, pulses, chocolate, and teas are rich sources of phytochemicals; however, the wide diversity of these compounds requires optimized extraction methodologies to further characterization (Aguilera, 2021).

Utilization of these compounds has become widespread to minimize occurrence of common non-communicable diseases in adults. Plant-based foods contain many phytochemical compounds along with nutrients such as proteins, fats, carbohydrates, vitamins, and minerals (Narzary et al., 2016). Scientifically, research is being undertaken to bring around limelight, the therapeutic properties of the phytochemicals present in these plants and also use them as a yardstick in modern medicinal plant uses. Phytochemicals are usually commented as research compounds rather than dietary nutrients because evident of their possible health effects has not been established yet.

Gomphrena globosa L. which has an abundance of phytochemicals and medicinal properties (commonly called Globe amaranth) belongs to the family Amaranthaceae, native to America

and spread widely in Asia. Globe amaranth has been identified in a variety of traditional medicine systems for the treatment of various human diseases. Figure 1 shows the picture plant and Table 1 provides its systematic position.



Figure 1: *Gomphrena globosa* L.

The leaves and flowers are used in folk remedy for oliguria, heat and empacho, hypertension, antimicrobial, antioxidant, cough, diabetes, hypertension, kidney problems, hoarseness, bronchitis and other respiratory diseases, mainly as expectorant, reproductive problems as well as it exerts significant cytotoxic and estrogenic activity (Latha et al., 2013). A major phenol was found to be kaempferol 3-O-rutinoside based on chromatographic and mass spectrometry techniques (Luis et al., 2012).

The pink globe amaranth hydromethanolic extract revealed the highest antioxidant activity, followed by red and white samples. The anti-inflammatory activity in red and pink varieties was more relevant. None of the samples showed toxicity to hepatic cells (Muhammad *et al.*, 2013). *Gomphrena globosa* is a popular edible plant used as food colorant and in traditional medicine. 24 phenolic compounds and eight betacyanins were determined by HPLC-DAD in three different extracts of *G. globosa* inflorescences. It has relevance in treating acute and chronic inflammatory conditions (Silva *et al.*, 2012).

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Table 1: Systematic Position

Kingdom	Plantae
Clade	Angiosperms
Order	Caryophyllales
Family	Amaranthaceae
Genus	<i>Gomphrena</i>
Species	<i>G. globosa</i> L

Gomphrena species have biological activities which were employed in folk medicine to treat oliguria, heat, hypertension, cough, diabetes, hoarseness and cough. It also has significant antimicrobial, cytotoxic and estrogenic use as analgesic for toothache. *G. globosa* extracts have also been used to treat jaundice, high cholesterol and urinary problems in Latin America and Caribbea (Roriz *et al.*, 2014). The objective of the study is to examine the phytochemicals of *Gomphrena globosa* flower extract and its antimicrobial activity.

2. MATERIALS AND METHODS

2.1. Collection of Plant Sample:

Pink Globe Amaranth flowers were collected from Krishnarajapuram, Bengaluru, Karnataka. The petals were detached carefully, washed thrice and kept for shade drying at room temperature for five days.

2.2. Preparation of Extract

The partially dried petals of *Gomphrena* were collected in a clean tray and weighed (560g). It was then kept into a hot air oven to remove any excess moisture or water content. The

dried petals were made into fine powder by grinding it in a kitchen blender for further analysis.

2.3. Solvent Extraction

The arid powdered petal samples of the *globosa* flower were used for the extraction of phytochemicals using the solvents namely Acetone, Methanol and Dimethyl Formamide (DMF). 20ml of each solvent were add up in three 250ml Erlenmeyer conical flask with these 5 grams of finely powdered dried petals were added and sealed. This mixture was then placed on a rotary shaker for about 2 days at room temperature to filtrate the extract for phytochemical Analysis.

2.4. Phytochemical Analysis of *Gomphrena* flower extract

Phytochemical screening of solvent extracts of *Gomphrena* flower were subjected to preliminary phytochemical screening

2.4.1 Mayer's Test for Alkaloids: A small quantity of the extract was treated with few drops of dilute hydrochloric acid and filtered. Then it was tested with alkaloid Mayer's reagent to observe the creamy precipitation to confirm the presence of alkaloids.

2.4.2 NaOH Tests for Flavonoids: To 2-3 ml of extract, few drops of sodium hydroxide solution were added in a test tube. Deep yellow colour on addition of few drops of dilute Hcl indicates the presence of flavonoids.

2.4.3 Phenol Test: When 0.5 ml of FeCl₃ Phytosterols solution was added to 2 ml of test solution, formation of an intense colour indicates the presence of phenols.

2.4.4 Foam Test for Saponins: The extract was diluted with 20 ml of distilled water and it was shaken in a graduated cylinder for 15 minutes. A thin layer of foam indicates the presence of saponins

2.4.5 Test for Anthocyanin: 0.5mg of extract was mixed with 2-3 drops of concentrated HCL in a test tube. Pinkish Red color solution indicated the presence of Anthocyanin.

2.4.6 Test for Terpenoids: 0.5mg of extract was mixed with 0.5ml of chloroform and a drop of sulfuric acid in a test tube to observe reddish brown precipitate.

2.4.7 Test for Glycosides: 1mg of extract was mixed with 1ml of glacial acetic acid and a drop of ferric chloride in a test tube. Sulfuric acid is added along the sides of the tube to observe the formation of brown ring.

2.4.8 Test for Quinones: 2.5mg of extract was mixed with 250 microlitre of isopropyl alcohol and a few drops of sulfuric acid in a test tube to observe the formation of brown solution.

2.5. Preparation of media for culturing microorganisms

3.12 gm of Potato dextrose agar (PDA) was dissolved with 80 ml of distilled water in a clean dry conical flask. The mixture in the conical flask was sealed with sterile cotton plug and left for sterilization in an autoclave at 121°C for about 15 minutes. Then 100 micrograms of antibiotic streptomycin were mixed to the media to avoid the growth of bacteria and then 20ml of the sterilized media was aseptically transferred to sterilized petri

plates and allowed to solidify. Similarly, 50 gm of Muller Hinton Agar was dissolved with 50 ml of distilled water in a clean dry conical flask. The mixture in the conical flask was sealed with sterile cotton plug and left for sterilization in an autoclave at 121°C for about 15 minutes then 20ml of the sterilized media was aseptically transferred to sterilized petri plates and allowed to solidify.

9.75 gm of Sabouraud dextrose agar (SDA) was dissolved in 250 ml of distilled water. The nutrient in the flask was tightly sealed and kept for sterilization in the autoclave at 121°C for about 15 minutes. Then 100 micrograms antibiotic Streptomycin was mixed to avoid the growth of bacteria. The sterilized medium was poured in sterile petri plates and were allowed to solidify.

2.6. Isolation and Identification of Fungi

Infected leaves of tomato and guava were collected and inoculated on potato dextrose agar media in sterile petri plates. After 9-10 days of incubation the fungus was isolated and morphologically confirmed using Lacto Phenol Cotton Blue stain.

2.7. Estimation of Antifungal Activity

The antifungal activity of Globe Amaranth flower extract was determined by Agar Well Diffusion method against *F. solani*, *F. oxysporum* and *A. brassicicola* with different concentrations. For that, 100 microlitre of spore suspension of these three isolated fungi was added to the sterilized Sabouraud dextrose agar media with the help of gel puncture at various concentrations like 0, 100, 150, 200 microgram/microlitre. The plates were incubated at room temperature and observed for the inhibitory effect of the extracts on fungal species maintained in triplicates.

2.8. Estimation of Antibacterial Activity by broth micro dilution method

To identify the lowest concentration required for a given antibiotic to inhibit bacterial growth, and 100 microlitre of bacteria is introduced into wells of liquid media containing progressively lower concentrations of the drug. The antimicrobial concentration is adjusted 200 microlitre by mixing stock antimicrobial with media. The adjusted antimicrobial is serially diluted into multiple tubes, inoculated and incubated for 16–20 hours. The antibacterial activity of *G. globosa* flower extract was determined by using the 96 well plates for finding the Minimum Inhibitory Concentration (MIC). In this method, 100 microlitre of the sterilized Mueller Hinton Broth is poured from the second well to the last well of the 96 well plates (A2-A12). 200 microlitre of the extract (dissolved again in DMSO) is then poured onto the first well (A1) then 100 microlitre is serially diluted (from A1- A11) and 100 microlitre is discarded from A11. The last well i.e. A12 remains the control for the experiment. A 100 microlitre of bacterial suspension is then poured into all the wells. The plate was then covered with the lid and kept for incubation at room temperature for 24 hours. After the period of incubation, the plate is then subjected to plate reader and the data is recorded.

3. RESULTS AND DISCUSSION

3.1. Identification of microbial isolates

From the inoculated plates of Potato Dextrose Agar and Sabouraud Dextrose Agar media, the fungi *Fusarium oxysporum*, *Fusarium solani* and *Alternaria brassicicola* were identified, subcultured and stored for further analysis (Figure 2).

3.2. Phytochemical Analysis of Gomphrena

The phytochemical screening of *globosa* flower extract using Methanol as a solvent confirmed the presence of phytochemicals, Flavonoid, Alkaloid, Saponins, Anthocyanin, Terpenoid, Phenol, and Glycosides. Acetone extract showed the presence of Flavonoid, Alkaloid, Anthocyanin, Terpenoid, and Phenol. Similarly, solvent Dimethylformamide (DMF) extract confirmed the presence of Flavonoid, Alkaloid, Terpenoid, Phenol and Glycosides (Table 2).

3.3. Antibacterial Assay

Antimicrobial assay of Globe Amaranth flower extract (*Gomphrena globosa*) is performed by Minimum Inhibitory concentration assays that determine the lowest concentration of an antimicrobial agent that prevents visible growth of any microorganism (Figure 3 & 4).

3.3.1 MIC of Methanol extract of *globosa* sample

Inhibitory concentration of methanol extract of *globosa* against *Bacillus subtilis* was found to be 0.294 followed by *Escherichia coli* 0.216 compared to the bacteria *Staphylococcus aureus* 0.367 at the concentration of 0.0976%.

3.3.2 MIC of Acetone extract of the *globosa* sample

Against the bacteria *Staphylococcus aureus*, the MIC was found to be 0.239 at the concentration of 0.0976% followed by the pathogen *Bacillus subtilis* 0.333 compared to *Escherichia coli*, the MIC was found to be 0.243 at the concentration of 0.1953%.

3.3.3 MIC of DMF extract of the *globosa* sample

Against the bacteria *Staphylococcus aureus*, the MIC was found to be 0.188 at the concentration of 1.562% followed by bacteria *Bacillus subtilis* found to be 0.328 at the concentration of 0.0976% compared to *Escherichia coli*, the MIC was found to be 0.266 at the concentration of 0.1953%.

4. CONCLUSION

Phytochemicals and their consumption strictly provides beneficial health effects. Preclinical and clinical findings recommend that phytochemicals may be effective in treating various diseases due to its antioxidant and anti-inflammatory properties. On the other side, consumption of few may led some acute and chronic toxic effects and may even cause cancer. It is obvious that the number of phytonutrients taken, the individuals age and gender, and the conditions, as well as exposure levels, is important in the occurrence of potential risks. Consumers need to know the right phytonutrients dose they should take in either foods or dietary supplements.

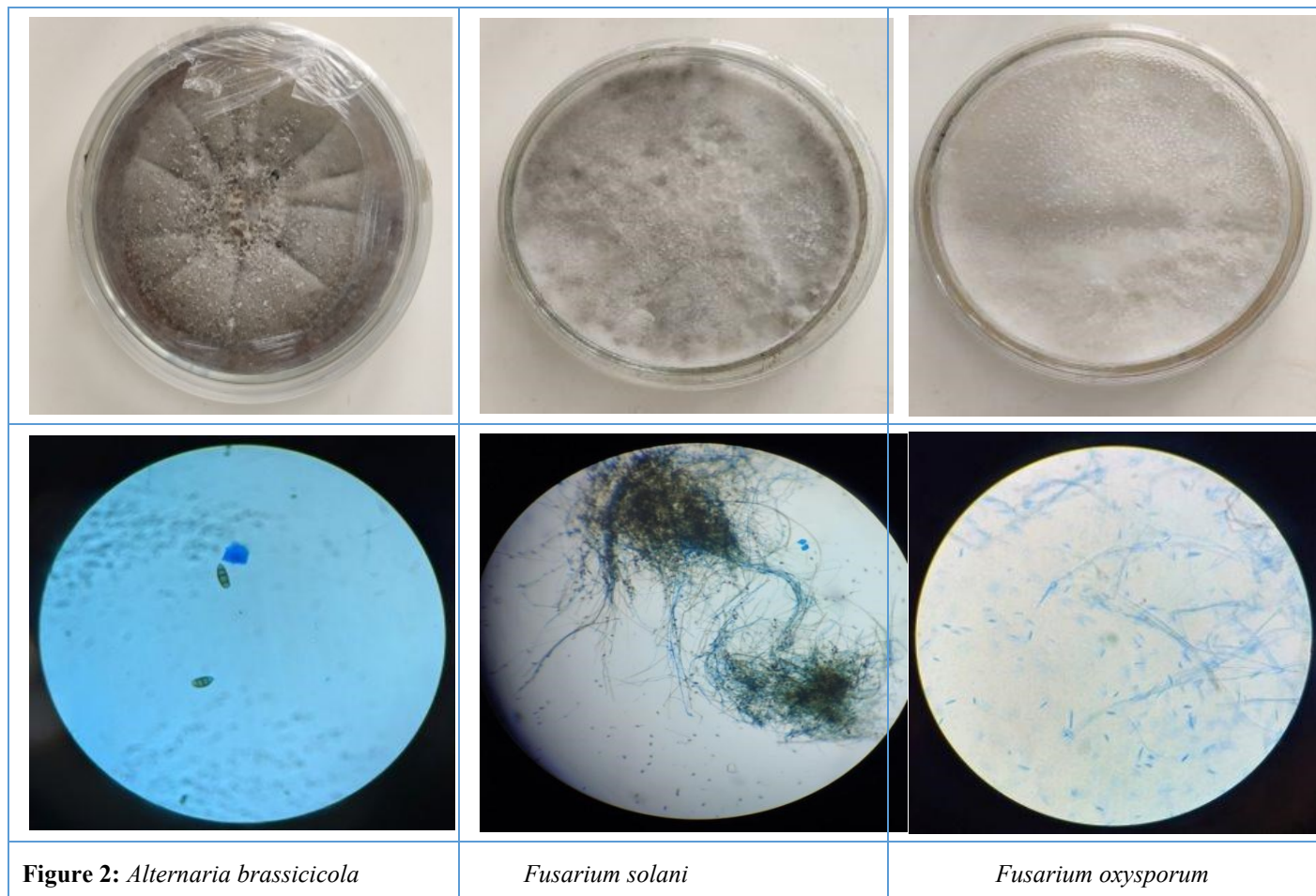


Table 2: Phytochemical analysis of Methanol, Acetone and DMF extracts of *globosa*

S.No.	Phytochemicals Tested	Extract of Methanol	Extract of Acetone	Extract Dimethyl Formamide (DMF)
1.	Flavonoid	+	+	+
2.	Alkaloid	+	+	+
3.	Saponins	+	-	-
4.	Anthocyanin	+	+	-
5.	Terpenoid	+	+	+
6.	Phenol	+	+	+
7.	Quinone	-	-	-
8.	Glycosides	+	-	+
Note:		+ Positive , - Negative		

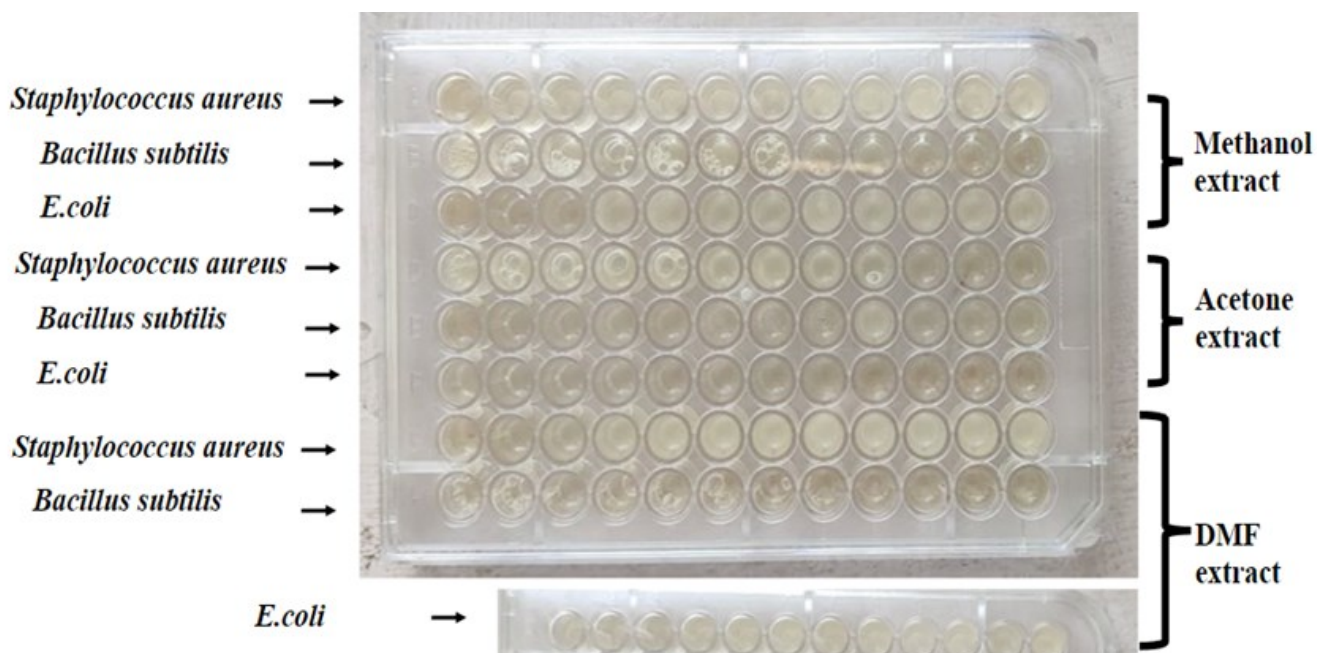


Figure 3: Broth Macrodilution Method

		Concentration %											
		100%	50%	25%	12.5%	6.25%	3.125%	1.562%	0.7812%	0.3906%	0.1953%	0.0976%	CONTROL
Optical Density 600nm	(METHANOL)												
	<i>Staphylococcus aureus</i>	0.137	0.138	0.170	0.238	0.287	0.363	0.374	0.327	0.267	0.361	0.367	0.437
	<i>Bacillus subtilis</i>	0.113	0.069	0.152	0.312	0.363	0.327	0.324	0.434	0.374	0.345	0.294	0.432
	<i>E. coli</i>	0.163	0.128	0.205	0.240	0.265	0.214	0.263	0.249	0.268	0.249	0.216	0.281
	(ACETONE)												
	<i>Staphylococcus aureus</i>	0.161	0.131	0.156	0.180	0.292	0.194	0.417	0.364	0.244	0.236	0.239	0.284
	<i>Bacillus subtilis</i>	0.165	0.075	0.113	0.330	0.342	0.320	0.300	0.366	0.334	0.322	0.333	0.353
	<i>E. coli</i>	0.136	0.113	0.164	0.208	0.254	0.257	0.233	0.230	0.238	0.243	0.276	0.272
	(DMF)												
	<i>Staphylococcus aureus</i>	0.254	0.239	0.183	0.162	0.196	0.180	0.188	0.412	0.386	0.426	0.448	0.353
	<i>Bacillus subtilis</i>	0.194	0.127	0.125	0.309	0.304	0.318	0.297	0.571	0.292	0.318	0.328	0.390
	<i>E. coli</i>	0.170	0.155	0.251	0.255	0.269	0.255	0.249	0.249	0.493	0.254	0.266	0.273

Figure 4: MIC data recorded by plate reader

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