

***In vitro* Antioxidant and Antibacterial Potential of Leaf and Stem of *Gloriosa superba* L.**

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ABSTRACT

In the present study, leaf and stem of *Gloriosa superba* L. were selected to evaluate its antioxidant and antibacterial potential. The extraction was done by a cold percolation method using solvents like hexane, ethyl acetate and methanol. The antioxidant activity was evaluated using antioxidant assays like superoxide radical scavenging assay, Ferric reducing antioxidant power and reducing capacity assessment. Total phenol and flavonoid content was measured by Folin- ciocalteu's reagent and aluminium chloride colorimetric method respectively. The antibacterial activity was done by an agar well diffusion method against four Gram positive and four Gram negative bacteria. The methanol extract of leaf and stem showed highest FRAP activity and highest reducing capacity assessment respectively. There was a direct correlation between total phenolic content and antioxidant activity. Gram positive bacteria were more susceptible than Gram negative bacteria. Methanolic extract of leaf showed best antioxidant and antibacterial activity and can be further exploited as a natural source of antioxidant and antimicrobial.

Keywords: *Gloriosa superba*, antioxidant activity, antibacterial activity, leaf, stem.

INTRODUCTION

India is a veritable emporium of medicinal and aromatic plants. It has been estimated that out of 15,000 higher plants occurring in India, 9,000 are commonly useful, of which 7,500 are medicinal, 3,900 are edible, 700 are culturally important, 525 are used for fiber, 400 are fodder, 300 for pesticide and insecticide, 300 for gum, resin

and dye and 100 for incense and perfume. In terms of the plant materials for traditional medicine, it is estimated that local communities has used over 7,500 plants species¹. Herbal medicines are in great demand in the developed and developing countries for primary health care because of their efficacy, safety and lesser side effects.

India has rich traditional knowledge, heritage and large biodiversity of medicines has a dismal share of the world markets due to export of crude extract and drugs².

Polyphenolic compounds are commonly found in both edible and inedible plants, and they have been reported to have multiple biological effects, including antioxidant activity. Flavonoids are plant phytochemicals and the six classes of flavonoids (flavonones, flavones, flavonols, isoflavonoids, anthocyanins and flavans) vary in their structural characteristics around the heterocyclic oxygen ring. Structurally, flavonoids are usually characterized by a C&S carbon skeleton. Flavonoids occur as aglycones (without sugar moieties) and glycosides (with sugar moieties).

Oxidative stress is an imbalance between reactive oxygen species (ROS) production and the intracellular capacity for removing ROS. It is leading to excessive damage of all bio molecules like DNA, RNA, lipids, proteins micronutrients like carotenoids, vitamins, etc³. The oxidation induced by free radicals result in cell membrane and membrane protein disintegration and mutation, which can further initiate the development of many diseases such as ageing, atherosclerosis, lipofuscinosis, oxygen toxicity and liver injury^{4,5}. Cancer and Cardiovascular diseases^{6,7}. Free radicals not only cause human disease but also cause lipid oxidation in the food system. Oxidation of lipids, which is the main cause of quality deterioration in many food systems lead to the development of undesirable off-flavors and formation of some toxic compounds and lowers the quality and nutritional value of food.

Antioxidants are compounds which inhibit oxidation, or free radicals induced oxidative damage and therefore are potential quenchers of free radicals or reactive oxygen species. Antioxidants act by one or more of

the following mechanisms: reducing free radical activity, scavenging free radicals, potential complexing of pro-oxidant metals and quenching of singlet oxygen⁸. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate (PG) have been widely used around the world for decades⁹ but they have restricted use in food products as they are suspected to be carcinogenic¹⁰.

Bacterial diseases accounts for a high proportion of health problems in the developing countries. To manage the bacterial diseases, many synthetic antibiotics are regularly used. Due to indiscriminate use of synthetic antibiotics, bacteria have developed resistance to many antibiotics and as a result, immense clinical problems in the treatment of infectious diseases have been created¹¹. This ongoing emergence of multi-drug resistant bacteria and the infectious diseases caused by them are serious global problem¹². Thus, there is an urgent need for novel antimicrobials or new approaches to combat these problems^{13, 14}.

Gloriosa superba L. (Glory lily) is an important medicinal plant belonging to the family Liliaceae. It is known as Creeping lily or Flame lily, is a native of tropical Africa and is also found in tropical Asia, including Bangladesh, India, Sri Lanka,



Malaysia and Myanmar¹⁵. It is widely used as a medicinal plant in south India, despite the fact that the whole plant is very poisonous².

It is one of the endangered species among the medicinal plants¹⁶. It is a semi-woody herbaceous branched climber, reaching approximately 5 m in height. One to four stems arise from a single V-shaped fleshy cylindrical tuber. It is one of the major medicinal plants in India cultivated for its seeds which are exported to developed countries for pharmaceutical use. Different parts of the plant have a wide variety of uses, especially within traditional medicine practiced in tropical Africa and Asia. The tuber is used traditionally for the treatment of bruises and sprains, colic, chronic ulcers, hemorrhoids, cancer, leprosy and also for inducing labor pains. Because of its similar pharmacological action, the plant is sometimes used as an adulterant of aconite (*Aconitum* sp.). In the world market glory lily is considered as a rich source of colchicines and gloriosine¹⁷. Seeds and tubers contain valuable alkaloids *viz.*, colchicine and colchicoside as the major constituents, which are used to treat gout and rheumatism. Due to the action of colchicoside on spindle fiber formation during cell division, the plant has been identified as a potential anti-cancer drug. Colchicine is a powerful antimitotic agent that blocks or suppresses cell division by inhibiting mitosis, the division of a cell's nucleus. Paste of the tuber is externally applied for parasitic skin diseases¹⁸. In the Indian Systems of Medicine, the tubers are used as tonic, antiperiodic, antihelmenthic and also against snake bite¹⁹. Medicinally, the tuber is used as an abortifacient, and in smaller doses it acts as a tonic, stomachic and anthelmintic. It is also fed to cows and goats in labour²⁰.

Thus, there is continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for the treatment of infectious diseases caused by multidrug-resistant microorganisms and stress related diseases and disorders. . In the present work *Gloriosa superba* L. was selected to evaluate its phytochemical, antioxidant and antibacterial properties.

MATERIAL AND METHODS

Collection of the plant material

The leaf and stem of *Gloriosa superba* Linn were collected in August 2013 from the Veraval, Junagadh, Gujrat, India. The plant was compared with a voucher specimen (PSN 728) deposited at Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. They were thoroughly washed, separated and dried under shade. The dried plant parts (leaf and stem) were crushed to fine powder and stored in airtight bottles which were later used for solvent extraction.

Extraction

The dry powder of leaf and stem of *G. superba* was individually extracted by cold percolation method²¹ using different organic solvents like Hexane (HE), Ethyl acetate (EA) and Methanol (ME). 10g of dried powder was taken in 100ml of Hexane in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24h. After 24h, the extract was filtered with eight layers of muslin cloth; centrifuged at 5000 rpm for 10 min. The supernatant was collected and the solvent was evaporated. The residue was then taken with 100 ml of solvent (ethyl acetate and methanol) in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24h. Then the procedure followed same as above, and the dry extract was stored in airtight bottles. The extract was weighed and the extractive yield was calculated.

Phytochemical analysis

Qualitative phytochemical analysis

The crude powder of leaf and stem of *G. superba* was subjected to qualitative phytochemical analysis^{22,23} to identify the presence or absence of different phytoconstituents like alkaloids, flavonoids, tannins, phlobatanins, triterpenes, steroids, saponins and cardiac glycosides.

Quantitative phytochemical analysis

Total phenol and flavonoid content were estimated in all the solvents extract of *G. superba* plant.

Determination of total phenol content

The amount of total phenol content of different solvent extracts was determined by Folin- ciocalteu's reagent method²⁴. The extract (0.5 ml and 0.1 ml of Folin-ciocalteu's reagent (0.5N) were mixed and the mixture was incubated at room temperature for 15 min. Then, 2.5 ml of sodium carbonate solution (2M) was added and further incubated for 30 min at room temperature and the absorbance was measured at 760 nm (Systronics, India), against a blank sample. The calibration curve was made by preparing Gallic acid (10 to 100 $\mu\text{g ml}^{-1}$) solution in distilled water. Total phenol content is expressed in terms of Gallic acid equivalent (mg g^{-1} of extracted compounds).

Determination of total flavonoid content

The amount of the flavonoid content of different solvent extracts was determined by aluminium chloride colorimetric method²⁵ the reaction mixture (0.3 ml) consisted of 1.0 ml of sample (1 mg ml^{-1}), 1.0 ml methanol, 0.5 ml of aluminium chloride (1.2%) and 0.5 ml potassium acetate (120 mM) and was incubated at room temperature for 30 min. The absorbance of all samples was measured at 415 nm using a digital spectrophotometer (Systronics, India), against a blank sample.

The calibration curve was made by preparing a quercetine (5 to 60 $\mu\text{g ml}^{-1}$) solution in methanol. The flavonoid content is expressed in terms of standard equivalent (mg g^{-1}) of extracting compound.

Antioxidant activities

The antioxidant activity of the different solvent extracts of *G. superba* was evaluated by superoxide anion radical scavenging activity ferric reducing antioxidant power and reducing capacity assessment.

Determination of superoxide (SO) anion radical scavenging activity

The antioxidant activity of the different solvent extracts of leaf and stem of *G. superba* was measured following the method described by Robak and Gryglewski²⁶ (1998). Superoxide radical are generated by oxidation of NADH and assayed by the reduction of NBT. The reaction mixture 3.0 ml consisted of 1.0 ml of the solvent extracts of different concentration of *G. superba* diluted by distilled water, 0.5 ml Tris-HCl buffer (16 mM, pH 8), 0.5 ml NBT (0.3 mM), 0.5 ml NADH (0.93 mM) and 0.5 ml PMS (0.12 mM). The superoxide radical generating reaction was started by the addition of PMS solutions to the mixture. The reaction mixture was incubated at 25 °C for 5 min and then the absorbance was measured at 560 nm using a digital spectrophotometer (Systronics, India), against a blank sample. Gallic acid (50 to 225 $\mu\text{g ml}^{-1}$) was used as a positive control. Percentage of inhibition was calculated.

Ferric reducing antioxidant power (FRAP)

The reducing ability of different solvent extracts of *G. superba* was determined by FRAP assay²⁷. FRAP assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of TPTZ, forming an intense blue Fe^{2+} -TPTZ complex with an absorption maximum at 593 nm. This reaction

is pH-dependent (optimum pH 3.6). 0.1ml extract is added to 3.0 ml FRAP reagent (10 parts 300 mM sodium acetate buffer at pH 3.6, 1 part 10 mM TPTZ (2,4,6- tripyridyl-s-triazine) in 40 mM HCL and 1 part 20 mM FeCl_3) and the reaction mixture is incubated at 37°C for 10 min. then the absorbance was measured at 593 nm. FeSO_4 (100 to 1000 μM Ml^{-1}) was used as a positive control. The antioxidant capacity based on the ability to reduce ferric ions of the sample was calculated from the linear calibration curve and expressed as M FeSO_4 equivalents per gram of extracting compounds.

Determination of reducing capacity assessment (RCA)

Reducing the capacity of different solvent extract was determined using the modified method of Athukorala *et al.*,²⁸. 1.0 ml of different concentration (20 to 180 mg ml^{-1}) of different solvent extracts and fraction diluted with distilled water, was mixed with 2.5 ml of phosphate buffer (200 mM pH 6.6) and 2.5 ml $\text{K}_3\text{Fe}(\text{CN})_6$ (30 mM). The mixture was then incubated at 50°C for 20 min. Thereafter, 2.5 ml of TCA (600mM) was added to the reaction mixture, and then centrifuged for 10 min. at 3,000 rpm. The upper layer of solution (2.5ml) was mixed with 2.5ml distilled water and 0.5ml FeCl_3 (6nm), and the absorbance was measured at 700 nm using a UV- VIS spectrophotometer (Shimadzu, Japan) against a blank sample. Ascorbic acid (20 to 180 μg ml^{-1}) was used as positive control.

Antimicrobial susceptibility testing

Test microorganisms

The microorganisms used were obtained from the National Chemical Laboratory, Pune, India. The microorganisms were maintained at 4°C. The Gram-positive bacteria studied were *Bacillus cereus* (BC) ATCC11778, *Staphylococcus aureus* (SA) ATCC29737, *Listeria monocytogenes* (LM)

ATCC19112 and *Corynebacterium rubrum* (CR) ATCC14898. The Gram-negative bacteria were *Escherichia coli* (EC) NCIM2931, *Pseudomonas aeruginosa* (PA) ATCC27853, *Klebsiella pneumoniae* (KP) NCIM2719 and *Salmonella typhimurium* (ST) ATCC23564.

Screening for antibacterial activity (Agar well diffusion assay)

In vitro antibacterial activity of different solvent extracts of leaf and stem of *G. superba* was determined by standard agar well diffusion assay^{29,30}. Mueller Hinton agar and Sabouraud dextrose agar media were used for antibacterial and antifungal activity respectively. Molten Mueller Hinton agar / Sabouraud dextrose agar (40-42°C) were seeded with 200 μl of inoculums (1×10^8 cfu/ml) and poured into Petri dishes. The media were allowed to solidify and wells were prepared in the seeded agar plates with the help of a cup borer (8.5 mm). Different extracts were dissolved in 100% DMSO at a concentration of 20 mg/ml, from this 100 μl of different extracts were added into the sterile 8.5 mm diameter well. The plates were incubated at 37°C and 28 °C for 24 and 48 h for bacteria and fungi, respectively. DMSO was used as a negative control. Antibacterial activity was assayed by measuring the diameter of the zone of inhibition formed around the well in millimeters. The experiment was done in triplicate and the average values were calculated for antibacterial activity.

RESULT AND DISCUSSION

Phytochemicals play an important role in the treatment of different types of diseases and disorders and are still used in both traditional and modern medicine. They are synthesized by specific biochemical pathways, for plant defence and adaptation to environmental stress. Bioactive compounds are generally accumulated as secondary

metabolites in all plant cells, but their concentration varies according to the plant part. The secondary metabolites range from medicinally useful agents to deadly poisons. Many of the secondary metabolites isolated from plants are used in pharmaceutical drug industry³¹.

In *G. superba* leaf, flavonoids, steroids and alkaloids by Mayer's reagent and Dragondroff's reagent were found in maximum amount. Tannins, triterpenes and alkaloids by Wagner's reagents showed good, but comparatively less content than flavonoids, steroids and alkaloids by Mayer's reagent and Dragondroff's reagent. Phlobatannins, saponins and cardiac glycosides were completely absent (Table 1). In *G. superba* stem, flavonoids, steroids, cardiac glycosides and alkaloids by Dragondroff's reagent were found in maximum amount. Triterpenes, alkaloids by Mayer's reagent and Wagner's reagent showed comparatively less content than flavonoids, steroids, cardiac glycosides and alkaloids by Dragondroff's reagent. Tannins, phlobatannins and saponins were completely absent (Table 1).

When leaf and stem of *G. superba* are compared, flavonoids were present in high amount in leaf as compared to stem. Tannins were present in leaf while, in the stem it was absent. Phlobatannins and saponins were absent in both the parts. Steroids were present in high amount in leaf as compared to stem. Cardiac glycosides were absent in leaf while in stem it was present in less amount. Triterpenes were present in high amount in leaf as compared to stem. Alkaloids by Mayer's reagent and Dragondroff's reagent were present in high amount in leaf as compared to stem. Alkaloids by Wagner's reagent were present in both the parts, but in less amount. On the whole, flavonoids were maximum followed by alkaloids in both the parts leaf and stem. The other phytoconstituents were part specific.

Phlobatannins and saponins were absent in both the leaf and stem.

Extraction yield

Efficiency of extraction is an important factor in the comparison of functional activity. Extractive yield depends on the choice of extraction method (cold percolation or Soxhlet method, successive or individual), extraction solvent (polarity). In the present work, individual cold percolation method was used for extraction. The extractive yield of different solvent extracts of *G. superba* leaf and stem are shown in Fig. 1. In leaf, maximum extractive yield was in the polar solvent ME extract (Fig. 1A). The extractive yield of non-polar solvent HE extract and semi-polar solvent EA extract was almost less. In stem, maximum extractive yield was also in polar solvent ME extract (Fig. 1B) while the extractive yield was moderate in semi-polar solvent EA extract and comparatively low in non-polar solvent HE extract.

When the extractive yield of both the parts is compared, maximum yield was in the ME extract in both the parts, while the yield of non-polar solvent HE extract was considerably more in leaf than in stem. The yield of semi-polar solvent EA extract was more on stem than in leaf. Extraction is critical for the recovery of phytochemicals, antimicrobials and antioxidants. The extraction yield depends on solvent, time and temperature of extraction as well as the chemical nature of the solvents. Extractive yield varies from plant to plant and different parts of the same plant. This is because of the variation in phytoconstituents and their concentration³². There are many reports in the literature where different solvent gave different extractive yield^{23, 33}.

Total Phenol and flavonoid Content

Phenolic compounds are secondary metabolites with many beneficial biological

effects like antibacterial, anti-inflammatory, antiallergic agents, antioxidant, antimutagenic as well as ability to modify gene expression³⁴. Flavonoids are the largest group of naturally occurring phenolic compounds which occur in different plant parts like root, stem, leaf, flower and peel, both in free and glycosides, they are known to be responsible for the antioxidant property of medicinal plant and are known for many biological activities³⁵. Folin-Ciocalteu reagent, a mixture of phosphotungstic and phosphomolybdic acids, is reduced to blue oxides of tungsten and molybdenum during phenol oxidation. This reaction occurs under alkaline condition provided by sodium carbonate. The intensity of blue color reflects the quantity of phenolic compounds, which can be measured using spectrophotometer³⁶. In the present work, total phenol and flavonoid content of the EA and ME extract of both leaf and was measured.

The total phenol content EA and ME extracts of leaf and stem of *G. superba* is shown in Fig. 2. The total phenol content of the leaf was more as compared to stem. The total phenol content of the leaf was more in polar solvent ME extract than the semi-polar solvent EA extract (Fig. 2A), while in stem the semi-polar solvent EA extract had more TPC than polar solvent ME extract (Fig. 2B). The total flavonoid content of EA and ME extracts of leaf and stem of *G. superba* is shown in Fig.3. The total flavonoid content was less in stem as compared to leaf. The total flavonoid content of the leaf was more in polar solvent ME extract than the semi-polar solvent EA extract, while in stem, the semi-polar solvent EA extract had more total flavonoid content than the polar solvent ME. The results suggest that extracting solvents significantly affected the phenol and flavonoid content as also suggested by (Almey *et al.*, 2010). He also reported different levels of total phenol content in *Polygonum minus*, *Murraya koenigii*, *Citrus*

hysrix and *Pandanus odurus* plants. There is a direct correlation between phenolic content and antioxidant activity of medicinal plants as reported by several researches^{37, 38}. Therefore, it is always essential to estimate the total phenol and flavonoid content before evaluating the antioxidant efficacy of any plant extract.

Antioxidant activity

Antioxidant can delay, inhibit or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. The plant kingdom offers a wide range of compounds exhibiting antioxidant activities, especially, plant polyphenols that have been investigated with the intent of finding compounds that can protect against a number of diseases related to oxidative stress and free radical induced damage³⁹. A number of *in vitro* antioxidant assays can be done to evaluate the antioxidant capacity of plant extracts. It is suggested that at least two antioxidant assays have to be performed to evaluate the antioxidant capacity of a plant⁴⁰. Hence, in the present work, three antioxidant assays were performed viz. super oxide anion free radical scavenging activity, FRAP and reducing capacity assessment.

Super oxide radical scavenging activity (SO)

The EA and ME extracts of leaf and stem of *G. superba* were evaluated for their super oxide radical scavenging activity. Their IC₅₀ values were determined. IC₅₀ value is the concentration of the extract which will scavenge 50% of the free radicals produced. Generally in antioxidant assays, IC₅₀ values of the plant extracts and the standards are calculated and compared. In the present work, the IC₅₀ values of both the solvent extracts of leaf and stem of *G. superba* was more than 1000 µg/ml (data not presented). Hence it can be concluded that these extracts

are not capable of scavenging the super oxide anion radicals.

Ferric reducing antioxidant power (FRAP)

The change in absorbance at 593 nm owing to the formation of a blue colored Fe (2)- tripyridyltriazine compound from colorless oxidized Fe (3) is formed by the action of electron donating antioxidants. This represents an electron exchange reaction. The calibration curve of ferrous sulphate (100-1000 μ M) was used, and results are expressed in μ mol Fe²⁺ /mg of extract (FRAP value). FRAP of EA and ME extracts of leaf and stem of *G. superba* is shown in Fig. 4. FRAP was less in stem as compared to leaf. The FRAP of the leaf was more in polar solvent ME extract than in the semi-polar solvent EA extract (Fig. 4A). In stem, the semi-polar solvent EA extract had slightly more FRAP activity than in the polar solvent ME extract (Fig. 4B).

Reducing power assessment (RCA)

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants. Reducing capacity assessment of EA and ME extract of leaf and stem of *G. superba* is shown in Fig. 5. In *G. superba* leaf reducing capacity was good in both leaf and stem. The reducing capacity assessment was less in both semi-polar solvent EA extract and polar solvent ME extract as compared to standard. In leaf, ME extract was very near to the standard and EA extract was good but less than methanol extract (Fig. 5A). In stem, the reducing capacity of ME extract was also very near to the standard and reducing the capacity of EA was good but less than ME extracts (Fig. 5B). Both the antioxidant

activities were moderate in both the parts of the plant. It appears that there is a need to evaluate other antioxidant assays and perhaps in other solvent extracts.

Antibacterial activity

In present study different solvent extracts of leaf and stem of *G. superba* were investigated for their antibacterial potential against four Gram positive and four Gram negative clinically important microbial strains. Antibacterial activity of HE, EA and ME extract of leaf and stem of *G. superba* against Gram positive bacteria are shown in Fig. 6. In *G. superba* plant leaf, non-polar solvent HE extract and polar solvent ME extract showed no activity against any of the four Gram positive bacteria. *B. cereus* and *S. aureus* was inhibited by semi-polar solvent EA extract. *L. monocytogenes* and *C. rubrum* were not inhibited by any of the three organic solvent extracts (Fig.6A). In *G. superba* plant stem, *B. cereus* was inhibited by all the three solvent extracts, while *S. aureus* was inhibited by only a polar solvent ME extract. *L. monocytogenes* and *C. rubrum* were not inhibited by any of the three organic solvent extracts (Fig. 6B).

Antibacterial activity of HE, EA and ME extract of leaf of *G. superba* against Gram negative bacteria is shown in Fig. 7. In leaf, *K. pneumoniae* was inhibited by non-polar solvent HE extract and semi-polar solvent EA extract. *E. coli*, *S. typhimurium* and *P. aeruginosa* were not inhibited by any of the three organic solvent extracts (Fig. 7). In stem, *E. coli*, *S. typhimurium* and *P. aeruginosa* were not inhibited by any of the three solvent extracts. The results suggest that HE, EA and ME extracts of leaf and stem of *G. superba* showed moderate but slightly more activity towards Gram positive bacteria than Gram negative bacteria. *L. monocytogenes* and *C. rubrum* were most resistant Gram positive bacteria and *E. coli*, *S. typhimurium* and *P. aeruginosa* were most

resistant Gram negative bacteria. *B. cereus* and *K. pneumoniae* were the most susceptible Gram positive and Gram negative bacteria respectively.

Generally polar solvent extracts of medicinal plants show more antibacterial activity as compared to non-polar or semi-polar solvent extracts⁴¹. But our results are contradictory. All the three extracts almost showed similar activity. In fact nonpolar solvent, HE extract of leaf showed more activity against *B. cereus* and *K. pneumoniae* than EA and ME. ME root extract of *Cissampelos pareira* L. had a broad spectrum activity by inhibiting both Gram positive and Gram negative bacteria⁴².

Finally, it can be concluded that the phenol and flavonoid content was maximum in methanol extract of leaf. The methanolic extract of leaf and stem showed maximum FRAP activity and reducing capacity assessment respectively. There was a direct correlation between total phenol content and antioxidant activity. The methanolic extract showed moderate antibacterial activity. Gram positive bacteria were more susceptible than Gram negative bacteria. Amongst both the parts and solvents used, methanolic extract of leaf showed best antioxidant and antibacterial activity. This can be a promising source of natural antibacterial and antioxidant agents, but further studies are needed with more solvents and more antioxidant assays. Work in this direction is in progress.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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Table 1. Qualitative phytochemical analysis of dried powder of leaf and stem of *G. superba*

S. No.	Test	<i>G. superba</i> leaf	<i>G. superba</i> stem
1	Flavonoids	+++	++
2	Tannins	++	-
3	Phlobotannins	-	-
4	Saponins	-	-
5	Steroids	+++	++
6	Cardiac glycosides	-	++
7	Triterpenes	++	+
8	Alkaloids		
9	Mayer's reagent	+++	+
10	Dragondroff's reagent	+++	++
11	Wagner's reagent	+	+

Phytochemicals present in less (+), moderate (++) and high (+++) amount; absent (-)

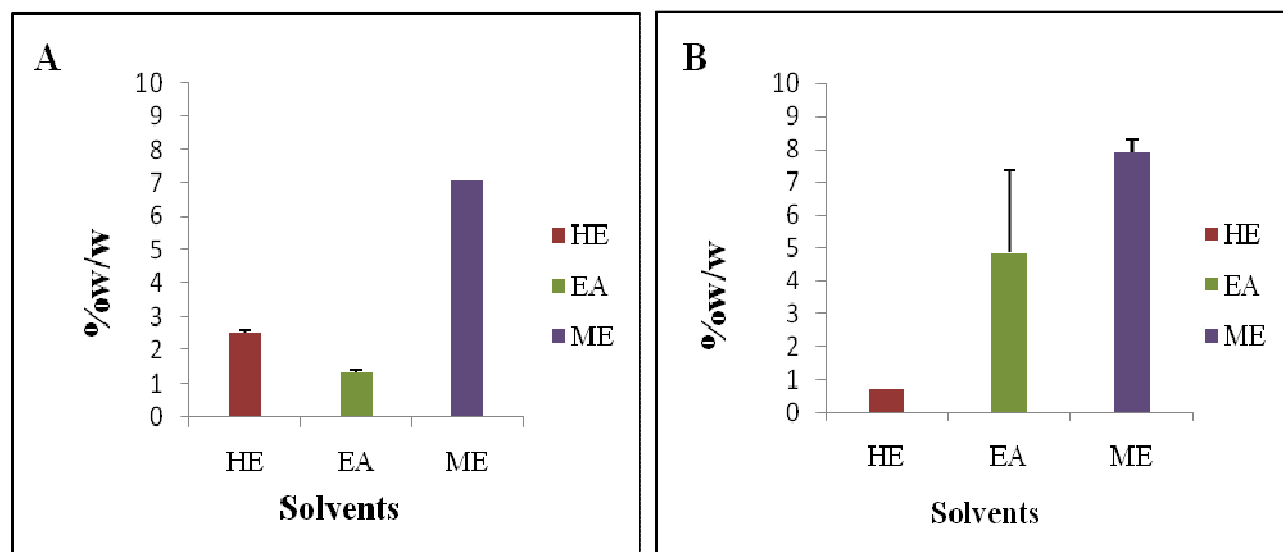


Figure 1. The extractive yield of different solvent extracts of *G. superba* leaf (A) and stem (B)

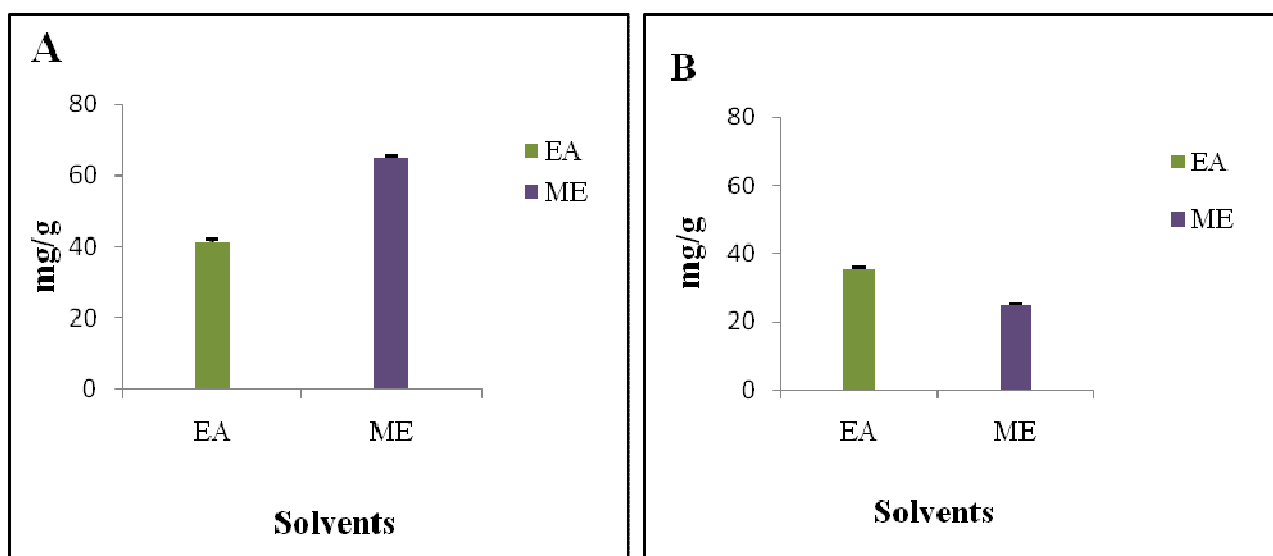


Figure 2. Total phenol content of ethyl acetate extract and methanol extract of *G. superba* leaf (A) and stem (B)

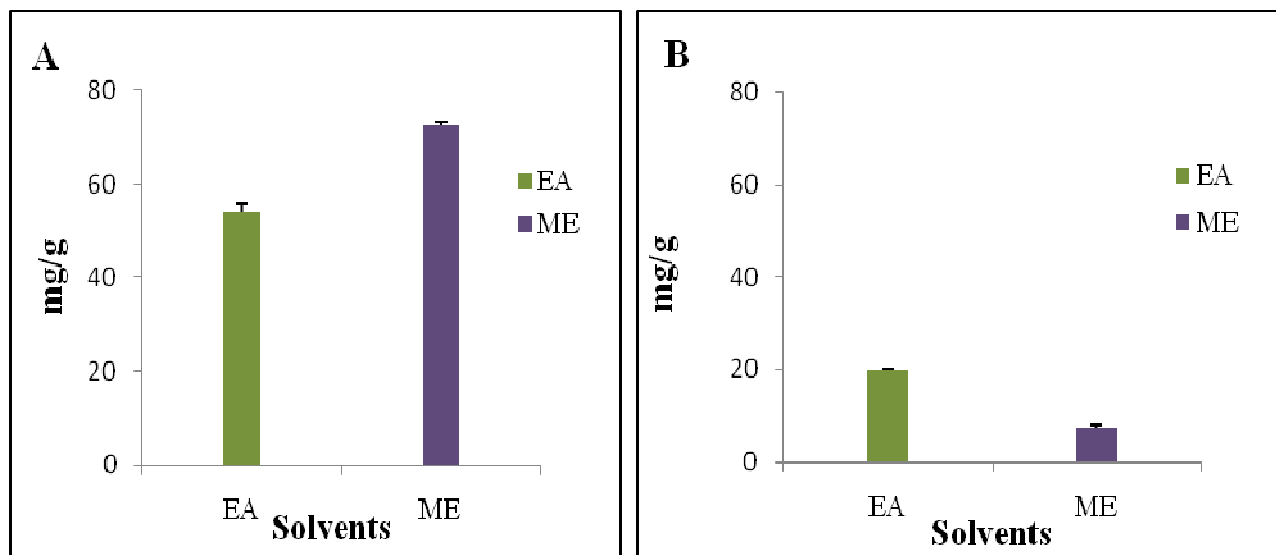


Figure 3. Total flavonoid content of ethyl acetate extract and methanol extract of *G. superba* leaf (A) and stem (B)

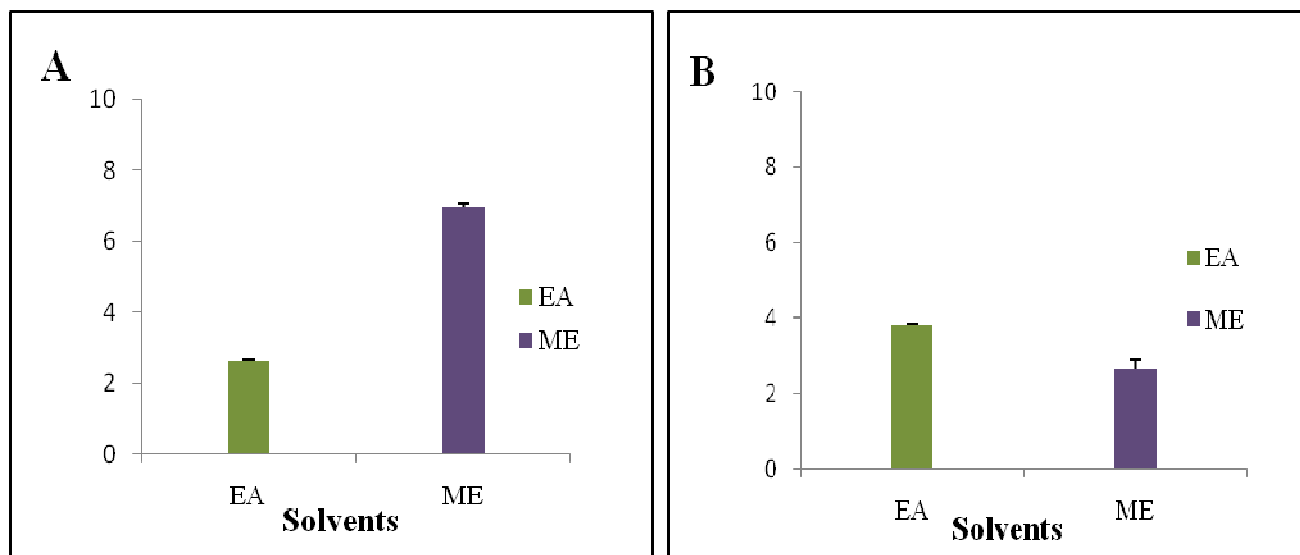


Figure 4. FRAP of ethyl acetate extract and methanol extract of *G. superba* leaf (A) and stem (B)

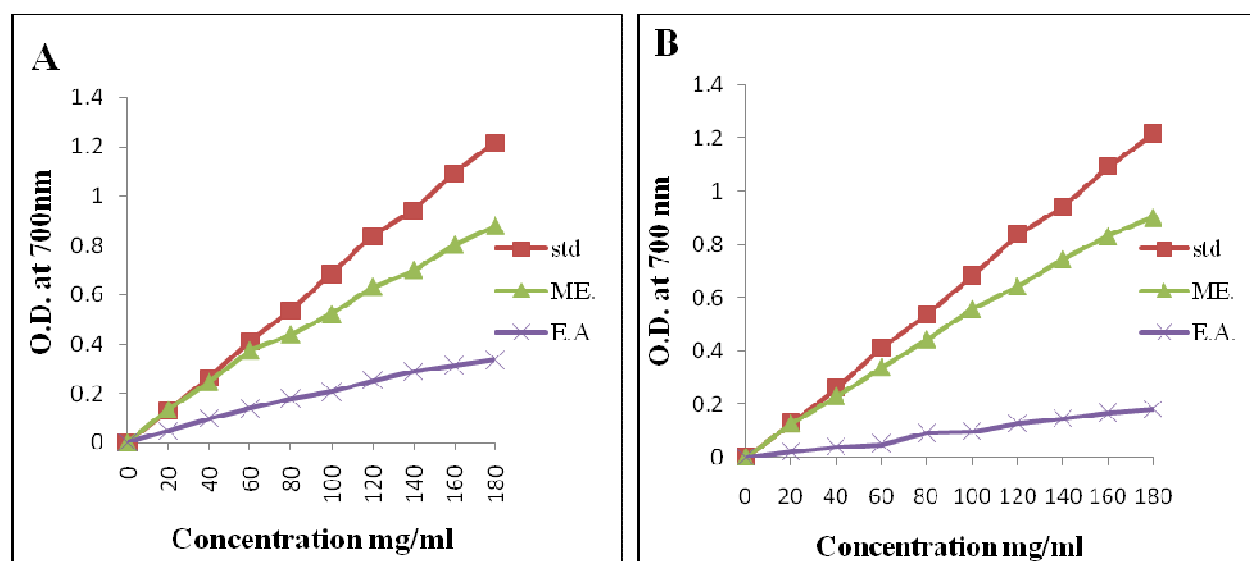


Figure 5. Reducing capacity assessment of ethyl acetate extract and methanol extract of *G. superba* leaf (A) and stem (B)

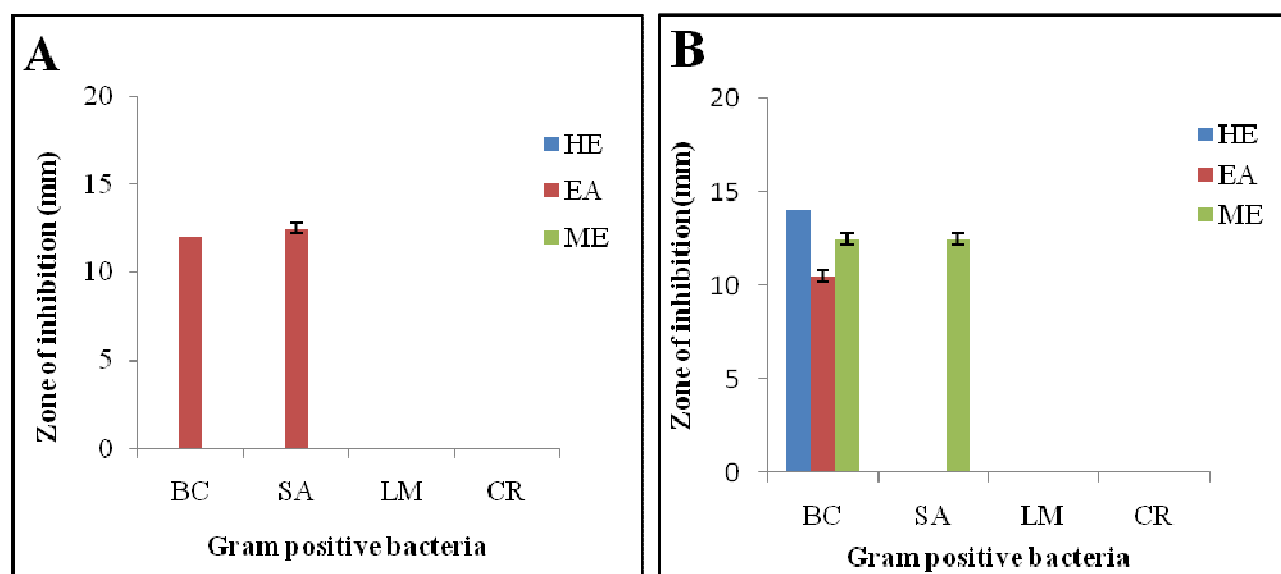


Figure 6. Antibacterial activity of different solvent extracts of *G. superba* leaf (A) and stem (B) against Gram positive bacteria.

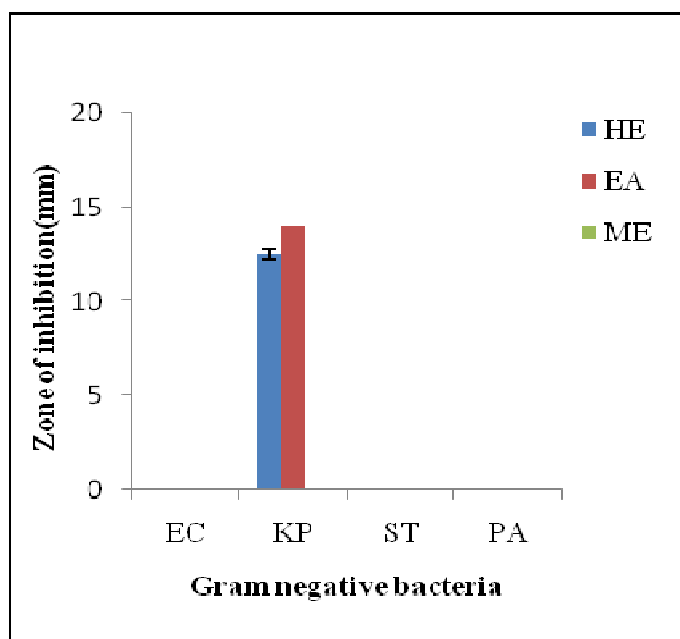


Figure 7. Antibacterial activity of different solvent extracts of *G. superba* leaf against Gram negative bacteria.