vaginal emission, leprosy, labor pain and abortion [13]. Injuries, skin problems, flu, inflammation, piles, blood disorders, lung contractions, general body toner, poison [11]. The major aim of this work was focused to perform phytochemical and antimicrobial activities on the whole plant extracts of *G. superba.*

MATERIALS AND METHODS

Plant materials

The fresh whole plants (leaves, stem, flower, and tuber) were collected from Bharathiar University, Coimbatore 2013–2014, Tamil Nadu, India. This plant was identified and recognized from the Department of Botany, Annamalai University, Tamil Nadu, India. All new plants collected and separated were then washed out in tap water, dried in the shade and finely powdered, and stored in air-tight container.

Chemicals

All chemicals were purchased by the SD Chemicals Company, Mumbai, and all chemicals were analytical grade.

Phytochemical screening

The bioactive compounds analysis of the dimethyl sulfoxide (DMSO), ethyl acetate (ETOAC), and ethanol (ETOH) of a flame lily whole plant extract was analyzed by standard methods as described [14-16]. The extract was analyzed for the presence of secondary metabolites (alkaloids, flavonoids, terpenoids, tannins, saponins, aromatic acids, phenolic compounds, xanthoproteins, triterpenoids, amino acids, philobatinins, carbohydrate, reducing sugar, and proteins).

Chemical group tests of the extract

Various chemical groups extracted were performed by phytochemical studies [14]. In each test, 10% (w/v) extraction solution was taken personally to be tested.

Active principle analysis

- Test for steroids and terpenoids: 9 ml ETOH was added to extract and refluxed to for a few minutes. Each filtrate was concentrated in boiling water to 2.5 mL. 5 mL of distilled water was added to a concentrated solution, and the mixture is allowed to stand for 1 h and the wax material is filtered. This filtrate was extracted using the separating funnel using 2.5 mL chloroform. 1 mL of concentrated H_2SO_4 was added to 0.5 mL chloroform extract in a test tube carefully. A reddish-brown interface showed steroids.
- The chloroform extract was evaporated and dehydrated to 0.5 ml on a water bath and heated with 3 ml of concentrated H_2SO_4 for 10 min on water bath. The presence of gray color showed terpenoids.

Test for flavonoids

About 20 mg of the extract from each of different solvents with 10 ml ETOAC is boiling water for 3 min. The mixture was filtered and the filtrate was used for the following tests:

- Ammonium test: 4 ml of the filtrate was shaken with 1 mL liquid ammonia solution (1%). The permitted layers were separated. A yellow color in the ammonia stack represented the presence of flavonoids.
- Test for alkaloids: The test solvent (0.2 g) was boiled through the 5 mL of 2% HCL on the water bath. The mixture was filtered and to 1 mL filtrate two drops of the following reagents were added:
  - Dragendorff's reagent: The formation of red precipitate confirms the presence of alkaloids
  - Wagner's reagent: The creation of reddish-brown precipitate indicated the presence of alkaloids
  - Hager's reagent: The development of yellow precipitates showed the presence of alkaloids.
  - Test for saponins: 500 mg of test solvent was boiled with 5 mL of distilled water for 5 min. The hot solution was filtered and the filtrate was utilized for further test:
  - Frothing test: 1 ml of the above filtrate was added with 4 mL distilled water, shaken vigorously, and observed for the appearance of stable froth.
- Test for tannins: 2 g test solution was heated with 5 mL of 45% ETOH for 5 min. The chilled solution was filtered and the filtrate was used for next test:
  - Lead-acetate test: 1 ml of filtrate was diluted with three drops lead acetate mixture and cream gelatinous precipitate showed the presence of tannins.
  - Ferric chloride test: 1 ml filtrate was added with distilled water and diluted and two drops of ferric chloride were added. Transient greenish to black color confirmed the presence of tannins.
  - Screening for phenols: To 1 ml extract and 3 mL of distilled water, few drops of 10% aqueous ferric chloride solution was added and the development of blue or green color showed the presence of phenols.
  - Macronutrient analyses: The test which would be shown subsequently was carried out to determine the presence of macronutrients in the climber of whole plant extract of *G. superba*.
  - Test for carbohydrate: 100 mg of the test solution was shaken vigorously with water, then filtered and the aqueous filtrate was mixed with few drops of Molisch's reagent, followed by concentrated H_2SO_4 (1 mL) to form a layer below the aqueous solution. A brown ring at the interface indicated the presence of carbohydrates.
  - Test for reducing sugar: 100 mg of the test solution was shaken vigorously with 5 ml of distilled water and filtered; the filtrate was mixed with equal volumes of Fehling solutions A and B then shaken vigorously. Brick red color precipitate confirmed the presence of reducing sugars.

Test for protein

Burette test

About 2 ml of test solution was place in a test tube, then five drops of 1% hydrated copper sulfate and 2 mL of 40% sodium hydroxide were mixed and shaken vigorously. A purple color indicated the presence of proteins (presence of two or more peptide bonds).

Antibacterial activity

Bacterial strains of *Salmonella abony, Pseudomonas aeruginosa, Micrococcus luteus, Staphylococcus aureus,* and *Escherichia coli* were used and maintained in nutrient agar (HiMedia, Mumbai) slants at 4°C. Mueller-Hinton agar (HiMedia, Mumbai) plates had been prepared, sterilized, solidified, and swabbed uniformly. *In vitro* antibacterial activity assay of aqueous DMSO, ETOAC, and ETOH was determined by way of agar well diffusion methods. The various concentrations of aqueous solvents 25, 75, and 125 µl have loaded into the wells of all plates. All bacterial strains are *S. abony, P. aeruginosa, M. luteus, S. aureus,* and *E. coli* obtained from Rajah Muthiah Medical College, Annamalai University, Annamalai Nagar. Area of inhibition changed into measured (mm) after incubating the plates at 37°C for 24 h. Triplicates have been maintained.

Fungal strains and inoculum quantification

The selected plant fungal pathogens *C. albicans* MTCC No. 7315, *C. krusei* MTCC No. 3020, *A. niger* MTCC No. 5889, *Rhizopus oryzae* (MTCC No. 3690), and *Mucor* Sp. MTCC No.3340 were obtained from the Department of Medical Microbiology, Rajah Muthiah Medical College and Hospital, Annamalai University, Tamil Nadu, India. The selected fungi are important among pathogenic fungi with economic impact to plants. The fungal strains were retained on potato dextrose (PD) agar. Amended hemocytometer cell counting was employed for estimating the number of cells in each fungal culture [17]. The inoculum preparation involved in the growth of fungus on PD agar slants for 1 week at 35°C. The slant was wiped off with a sterile cotton swap and shifted to a sterile tube with fresh PD broth (50 ml). The sterile tubes were then dazed for 5 min and appropriate dilutions were made to estimate the number of cells by microscopic enumeration with a hemocytometer. The final inoculums concentration was maintained to approximately 1.0×10^6 cells/ml.

Determining antifungal activity

Each well was filled with plant extracts (100 mL) serially diluted 50% and included by water in 96-well microtiter plates and 100 ml of fungal cultures were added [18]. The amphotericin B was used as an antibiotic reference and 100% acetone was a negative control. The previous concentration of the acetone in the microplate formerly showed [19] that the fungus did not have any influence on fungal growth. A display of growth 40 ml of 0.2 mg/ml p-iodonitrotetrazolium violet (INT) dissolved in water and filled to the microplate well. The enclosed microplate was incubated for 3–5 days in the plastic bag at 100% relative humidity at 35°C after sealing a plastic bag to minimize fungal contamination in the laboratory. The minimal inhibitory concentration was recorded as the lowest concentration of extract that could prevent the development of antifungal growth. Colorless tetrazolium salt acts as an electron receiver and is inhibited by a red-colored formazan developed with biological active organisms [18]. If the growth of the fungal was prohibited, a well-known solution is clear or a significant reduction in the severity of the color after it is attached to the INT.

Statistical analysis

Experiments have been performed in triplicates and the results have been expressed as mean ± standard deviation. The statistical analysis was made with origin software (OriginPro Evaluation, 2018).

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

The bioactive chemicals examined in the shoots, flowers, and tubers of *G. superba* whole plant extracts revealed the presence, absence, and not detected of compounds as listed (Tables 1 and 2).

The present studies confirmed that the presence of alkaloids, flavonoids, terpenoids, tannins, saponins, aromatic acids, phenolic compounds, xanthoproteins, triterpenoids, amino acids, carbohydrate, reducing sugar, and proteins was observed in tuber extracts while reducing sugars, xanthoproteins, amino acid, philobatinins, and proteins were absent. No amino acids were detected in shoot, flower, and tuber extracts of *G. superba*. The shoot and flower extract of *G. superba* showed negative results for terpenoids and saponins as reported in *Solanum torvum* [20]. The tuber extract showed positive results for terpenoids, alkaloids, saponins, and sterols, phenols as mentioned in sweet potato, *Asparagus racemosus*, elumpotti, *Ormocarpum cochinchinense*, Datura, *Camellia sinensis*, and *Guazuma ulmifolia* [21-28].

Antibacterial activity

Organic solvents DMSO, ETOAC, and ETOH of tuber extracts displayed more pronounced antibacterial activity than shoot and flower extract. Antibacterial activity extended with the increase inside the attention of the shoot, flower, and tuber extract. The maximum zone of inhibition (19.00±0.45 mm) was exhibited by ETOH tuber extract of *G. superba*. The DMSO, ETOAC, and ETOH tuber extract confirmed maximum area of inhibition in the various concentrations (25, 75, and 125 µL/ml) against shoot and flower as shown in Fig. 1.

This activity can be due to the potential ability of the secondary metabolites to shape a complex with extracellular proteins and with the cell wall of microorganism [29]. The inhibition effect of the methanol leaf extract, on the increase of bacteria, might be due to better solubility of these secondary metabolites, which showed the presence of a wide spectrum of antibiotic compounds [30]. The organic solvent of the plant extract (tuber) renders greater antibacterial ability than the

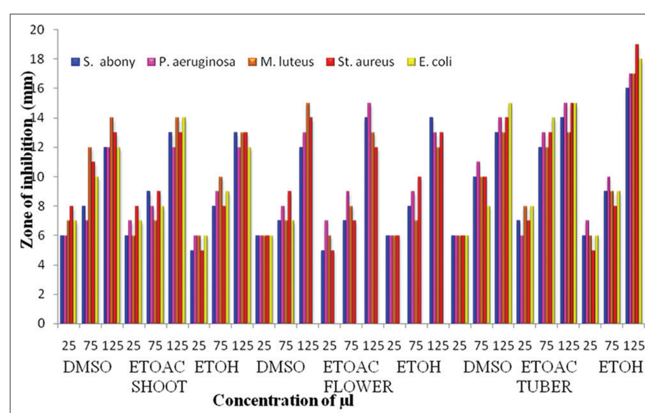

Fig. 1: Inhibition zone of three solvent extracts from shoot, flower, and tuber extracts of *Gloriosa superba* against five different bacterial pathogens

Table 1: Qualitative phytochemical analysis data in the shoot, flower, and tuber extracts of *G. superba*

S. No.	Parts of plant	Shoot			Flower				Tuber		
	Test for	DMSO	ETOAC	ETOH	DMSO	ETOAC		ETOH	DMSO	ETOAC	ETOH
1	Alkaloids	+	+	+++	+		+	+++	+	+	+++
2	Flavonoids	–	–	–	–		–	–	+	+	+++
3	Terpenoids	–	–	–	–		–	–	+	++	+++
4	Tannins	+	+	+++	+	+	+	+++	+	+	+
5	Saponins	–	–	–	–		–	–	+	+++	++
6	Aromatic acids	++	+++	+	–		–	–	+	+	+
7	Phenolic compounds	+	+	–	–		–	–	+	++	+++
8	Xanthoproteins	–	–	–	–	–		–	–	–	–
9	Triterpenoids	–	–	–	–		–	–	+	+	++
10	Amino acids	–	–	–	–		–	–	–	–	–
11	Philobatinins	Nd	Nd	Nd	Nd		Nd	Nd	Nd	Nd	Nd

– Absent, + low abundance, ++ Moderate abundance, +++ High abundances, Nd Not detected, DMSO: Dimethyl sulfoxide, ETOAC: Ethyl acetate, ETOH: Ethanol, *G. superba*: *Gloriosa superba*

Table 2: Macromolecule analysis data in the shoot, flower, and tuber extracts of *G. superba*

S. No.	Parts of plant	Shoot			Flower				Tuber		
	Test for	DMSO	ETOAC	ETOH	DMSO	ETOAC		ETOH	DMSO	ETOAC	ETOH
1	Carbohydrate	+	+	+++	+		+	++	+	+	+++
2	Reducing sugar	+	+	++	+		+	++	+	+	+++
3	Proteins	Nd	Nd	Nd	Nd		Nd	Nd	Nd	Nd	Nd

– Absent, + low abundance, ++ Moderate abundance, +++ High abundances, Nd Not detected, DMSO: Dimethyl sulfoxide, ETOAC: Ethyl acetate, ETOH: Ethanol, *G. superba*: *Gloriosa superba*

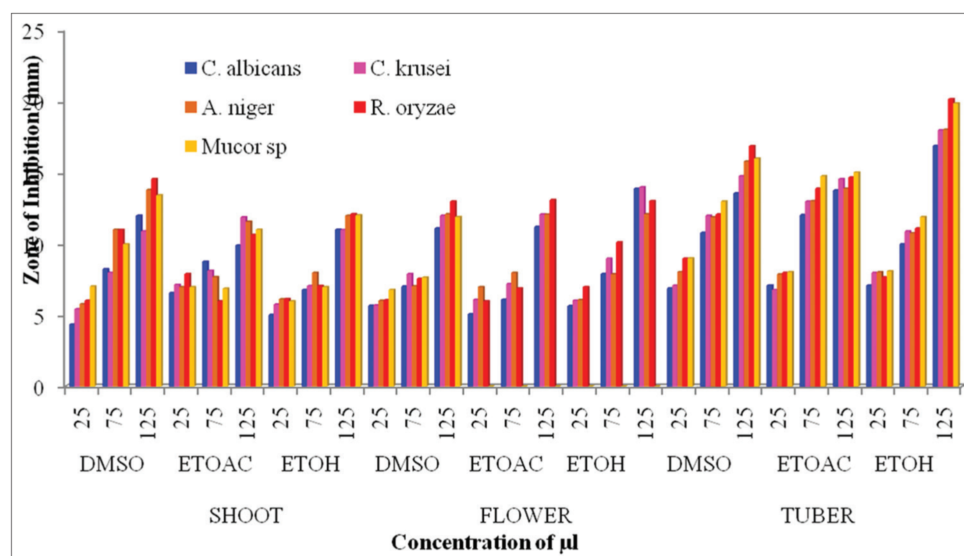

Fig. 2: Inhibition zone of three solvent extracts from shoot, flower, and tuber extracts of *Gloriosa superba* against six different fungal pathogens

shoot and flower extract. This result can be attributed to the polarity of the solvent used for extraction, essential bioactivity, and capability to diffuse in media utilized in assay [31,32]. Therefore, in many elements of the plant for herbal safety against microbial infection are packed with phytochemical substances. The phytochemical analysis revealed the presence of alkaloids, flavonoids, terpenoids, tannins, saponins, aromatic acids, phenolic compounds, xanthoproteins, triterpenoids, amino acids, carbohydrate, reducing sugar, and proteins in shoot, flower, and tuber extracts. Therefore, phytochemical products can be responsible for bacterial activity of *G. superba*.

Antifungal activity

The antifungal activity of the whole plant shoot, flower, and tuber extracts in different solvents was studied with the aid of agar well diffusion method against five pathogenic fungal species and measured the zone of inhibition for each fungal strain at various concentrations of 25, 75, and 125 µl/ml as shown in Fig. 2.

Antifungal activity of the tuber extracts of *G. superba* made with DMSO, ETOAC, and ETOH showed zone of inhibition against all the five fungal pathogens (*C. albicans, C. krusei, A. niger, R. oryzae,* and *Mucor* sp.) while none of the shoot and flower extracts made with three different solvents showed inhibition zones.

Herein, we report the antifungal activity of phytochemical extracts using DMSO, ETOAC, and ETOH from shoot, flower, and tuber of *G. superb.* It was found effective against *C. albicans, C. krusei, A. niger, R. oryzae,* and *Mucor* sp. In this study, it is observed that the zone of inhibition antifungal (*R. oryzae*) activities of shoot, flower, and tuber ETOH extract (14.06±0.57, 12.98±0.35, and 16.87±0.47 mm at 25, 75, and 125 µl/ml concentration, respectively) increased proportionately with the increase in the concentration of phytochemical extracts made using three different solvents from shoot, flower, and tuber of *G. superba*.

ETOHs solvent extracts of the tuber of *G. superba* showed highest inhibitory activity against selected fungi than DMSO and ETOAC. The current results confirmed with the previous study, DMSO, ETOAC, and ETOH tuber extract of *G. superba* which showed potential antifungal activity at 1 mg/disc against to *C. albicans, C. krusei, A. niger, R. oryzae,* and *Mucor* sp. [33]. The methanol and ETOH of whole plant extract showed potential antifungal activity against to *F. equiseti, B. theobromae,* and *C. corchori* [34]. The phytochemical screening revealed that plant secondary metabolites present in the extracts can agitate the fungal cell wall and also cause the discharge of cellular components such as ions and intracellular proteins which, in turn, stop the progress of the growth of fungi. The plant metabolites saponins, tannins, and alkaloids are known for antimicrobial activity which has been documented well [35]. Many scientists tried to explain the plausible mechanism of action of the antimicrobial activity of plant extracts. Triterpenoids or saponins present in the extracts could create pore-like structures and are responsible for the dissipation of membrane electrical potential or membrane proton motive force, and thus, membrane annihilation occurs [36].

CONCLUSION

Whole plant (shoot, flower, and tuber) extracts showed antibacterial and antifungal activity with maximum inhibition against to selected microorganisms. ETOHs of tuber exhibited maximum antibacterial and antifungal activity. Phytochemical screening revealed that alkaloids, triterpenoids, phenols, saponins, and flavonoids could be responsible for the antimicrobial activities of the *G. superba* whole plant extracts. It is evident from the current results that compounds of *G. superba* can be used as antimicrobial agents and ingredients in the human pathogenic diseased formulations in the different pharmaceutical fields.

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AUTHORS' CONTRIBUTIONS

TS conceived the idea, participated in designing, supervised, and monitored the work progress. UJ and AJ conducted the experiments; TS, DG, and UJ drafted the manuscript for publication. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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