CIBTech Journal of Microbiology ISSN: 2319-3867 (Online)
An Online International Journal Available at http://www.cibtech.org/cjm.htm
2014 Vol. 3 (2) April-June, pp. 48-57/ Nikhila et al.
Research Article

ANTIMICROBIAL SCREENING OF A MEDICINALLY POTENT PLANT - *GLORIOSA SUPERBA* L.

G .S Nikhila, G. Sangeetha and *T.S Swapna
Department of Botany, University College, Trivandrum 695034, Kerala, India
**Author for Correspondence*

ABSTRACT
Antimicrobial properties of *Gloriosa superba* was analysed in the present study. Tuber, flower and seed of the plant was collected separately, dried and powdered. Bioactive compounds from the fine powder of plant parts were extracted by distillation. Solvents used were petroleum ether, methanol, acetone, chloroform. For evaluation of antibacterial property, clinically and industrially important bacteria like *Bacillus species, Escherichia coli, Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Serratia marcescens and Proteus vulgaris* were selected. For antifungal study *Aspergillus niger, Rhizopus species, penicillium notatum, Trichoderma species, Neurospora crassa, Saccharomyces cerevisiae* and *Alternaria species* were used. Results showed that extracts from different plant parts has both antibacterial and antifungal effect. Among them methanol extract of tuber were found to be more active against all bacterial and fungal strains. So evidently there is scope for development of new antimicrobial agents using this plant.

***Keywords*:** *Bacillus species, penicillium notatum, Nutrient agar, Potato dextrose agar*

INTRODUCTION
Microorganisms are present in all habitats on the planet such as water, soil, acidic hot springs etc. and also present in plants and animals. The majority of microbes are harmless and beneficial to the organism. But some species are pathogenic and causes serious diseases. Antimicrobial agents are used to inhibit microbial growth. Several plants have this type of antimicrobial properties. In the present study antimicrobial properties of the *Gloriosa superba* were analysed.

Gloriosa superba L. is an important medicinal climbing herb belongs to the family Liliaceae (Ashok *et al*., 2011). The plant is mainly distributed throughout Africa and Asia. It is used to cure gout, cancer, asthma, arthritis, leprosy, piles, ulcers. (Ravindra *et al*., 2009). The high medicinal value is due to the presence of many secondary metabolites such as colchicine, colchicoside, gloriosine, chelidonic acid etc. (Rehana *et al*., 2012). All parts of *Gloriosa superba* have medicinal values. The leaf sap is used as a smoothening agent for skin eruptions (Joshi 1993). The V- shaped tuber is used for the treatment of cancer, haemorrhoids, chronic ulcers, leprosy and also for inducing labour pains. The tuber is also used as abortifacient, anthelmintic, tonic, stomachic and anti-inflammatory drug. Root tuber with sesamum oil will reduce the pain in arthritis affected joints (Abhishek *et al*., 2011). Seeds are used for curing rheumatic pain and also act as muscle relaxant. Colchicine is one of the important alkaloids mainly present in tubers and seeds of *Gloriosa superba* which is used to cure cancer and gout. Antifungal sensitivity of *Gloriosa superba* has been reported against *Candida albicans* and *Candida glaberata* (Haroon *et al*., 2008). Phytochemicals from root tubers have wide spectrum action against Gram- positive and Gram- negative bacteria along with antifungal and mutagenic potential. The phytochemicals from tubers has passed Ames *Salmonella* mutagenicity test due to the presence of Colchicine (Shanmugam *et al*., 2009).

MATERIALS AND METHODS
Plant material
The plant parts (tuber, flower and seed) of *Gloriosa superba* were collected separately and extracted by distillation process in Soxhlet apparatus. The solvents used were petroleum ether, methanol, chloroform and acetone.

Extraction

Forty grams of powder obtained from plant parts were covered with cotton cloth and kept in 100 ml of petroleum ether in Soxhlet apparatus. The apparatus was kept over a heating mantle and heated for 7-8 hour continuously at 60 to 70^0C. The extract was collected from the round bottom flask and kept open for 3 days for the complete evaporation of petroleum ether, until only the crude paste like extract remained in the beaker. The same procedure was done with the other three solvents such as methanol, chloroform and acetone.

Culture media and strains:

Nutrient agar medium and Potato dextrose agar medium were used for antibacterial and antifungal study respectively. Pathological strains such as *Bacillus species, Escherichia coli, Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Serratia marcescens and Proteus vulgaris* were used for testing antibacterial activity of the extracts. For antifungal study *Aspergillus niger, Rhizopus species, penicillium notatum, Trichoderma species, Neurospora crassa, Saccharomyces cerevisiae* and *Alternaria species* were selected.

Disc diffusion method

The antimicrobial activity of the extracts was determined by disc diffusion method. Disc of 4mm diameter were cut out from Whattman's No 1 filter paper. They were sterilized by autoclaving and stored in aseptic conditions in a test tube. During the time of treatment the disc was taken out from the test tube with the help of a forceps. The crude extract taken from the plant was pipetted out (20 mg) and poured into a clean autoclaved petri dish. Filter paper disc was placed in the petri dish for 20 minutes to make filter paper disc fully saturated with the extract. With the help of a forceps one filter paper disc was placed on nutrient media. Another filter paper was saturated with methanol and after 30 minutes it was placed on the media as a control. Likewise saturated filter papers with chloroform, acetone and petroleum ether were used as control. Gentamicin and Bavistin were used as standard in antibacterial and antifungal study respectively (Elgayyar *et al*., 2001)

After the spreading of microbes into the agar plate the disc was prepared with plant extract. The discs are impregnated with plant extract were placed in the plate containing pure culture and kept for incubation overnight. After one day zone of inhibition was noted.

RESULTS AND DISCUSSION

Antibacterial activity

The petroleum ether, methanol, chloroform and acetone extracts obtained from the tuber, flower and seed of *Gloriosa superba* were evaluated for antimicrobial property. All the extracts were showed antibacterial activity against all the selected organisms. The maximum inhibitory activity was seen in methanol extracts. In the case of tuber the high inhibitory activity seen in methanol extract against *Proteus vulgaris* and *Bacillus species* (Fig:1) and also in flower and seed maximum inhibition zone obtained in methanol extract against *pseudomonas aeruginosa* and *Staphylococcus aureus* respectively (Fig:2 & 3). To compare with the flower and seed, tuber has the highest antibacterial activity because tuber extract showed clear inhibition zone against *Proteus vulgaris* and *Bacillus species* than the standard (Table-1). The results were found to be in correlation with the previous studies (Senthilkumar *et al*., 2013) (Haroon *et al*., 2011) (Shanmugham *et al*., 2009). Previous studies revealed that the tubers and seeds of *Gloriosa superba* possessed good antibacterial activity in different solvents. Phytochemical screening and antimicrobial activity of *Gloriosa superba* were also reported against *Bacillus cereusm* (Senthilkumar *et al*., 2013). In the present study maximum antibacterial activity showed by methanolic tuber extract, followed by methanolic flower extract. At the same time methanolic seed extract of *Gloriosa superba* has least antibacterial activity.

Antifungal activity

All the extracts were showed antifungal activity against all the fungi. The methanol extract showed maximum inhibitory activity. In the case of tuber the high inhibitory activity seen in methanol extract

against *Rhizopus species* and *penicillium notatum* (Fig:4) and also in flower and seed maximum inhibition zone obtained in methanol extract against *Neurospora crassa* and *Saccharomyces cerevisiae* respectively (Fig:5 & 6). To compare with the flower and seed, tuber has the highest antibacterial activity since tuber extract showed clear inhibition zone against *Rhizopus species* and *penicillium notatum* than the standard (Table-2). The results are found to be in correlation with the earlier studies (Shanmugham *et al*., 2009) (Kamna *et al*., 2012) (Khan *et al*., 2008). These earlier studies revealed that the tubers and seeds of *Gloriosa superba* possessed good antifungal activity in different solvents. In the present study maximum antifungal activity showed by methanolic tuber extract, followed by methanolic flower extract. Seed of *Gloriosa superba* showed least antimicrobial activity than that of flower.

This antimicrobial property may be due to the presence of bioactive molecules and utilization of these potent compounds could be helpful for the production of new antimicrobial agent. This study will be helpful for the isolation and identification of new antimicrobial compounds for controlling the microbial pathogens and could be a valuable new drug source.

Table 1: Antibacterial studies of *Gloriosa superba* L.
Zone of inhibition in mm

Bacterial strains	*Standard	Tuber				seed				flower			
		PE	A	CH	ME	PE	A	CH	ME	PE	A	CH	ME
Salmonella typhi	19	5	14	11	16	5	6	6	8	5	9	6	13
Pseudomonas aeuriginosa	18	5	13	10	15	5	6	6	8	8	9	12	**16**
Staphylococcus aureus	22	5	12	10	19	6	9	6	**10**	5	6	7	13
Proteus vulgaris	22	5	16	8	**24**	6	7	7	8	6	7	11	12
Serratia marcescens	22	6	12	10	17	5	6	6	9	5	6	6	15
Escherichia coli	19	6	11	10	15	6	7	6	7	5	6	7	12
Bacillus species	19	5	14	9	**22**	5	6	6	7	5	5	7	12

***Standard-Gentamicin, PE- Petroleum ether, A- Acetone, CH- Chloroform, ME- Methanol*

Table 2: Antifungal studies of *Gloriosa superba* L.
Zone of inhibition in mm

Fungal strains	*Standard	Tuber				seed				flower			
		PE	A	CH	ME	PE	A	CH	ME	PE	A	CH	ME
Aspergillus niger	16	4	9	12	14	5	7	9	13	6	8	11	14
Rhizopus species	15	6	10	12	**18**	5	7	10	12	4	5	7	10
Penicillium notatum	18	6	10	13	**20**	4	6	7	11	5	12	9	16
Trichoderma species	7	3	7	10	12	4	5	6	7	2	3	4	5
Neurospora crassa	15	3	10	12	13	5	5	6	9	6	10	15	**19**
Saccharomyces cerevisiae	13	5	9	10	11	6	9	12	**16**	3	4	5	7
Alternaria species	14	4	5	7	9	4	7	8	12	5	7	10	12

***Standard-Bavistin, PE- Petroleum ether, A- Acetone, CH- Chloroform, ME- Methanol*

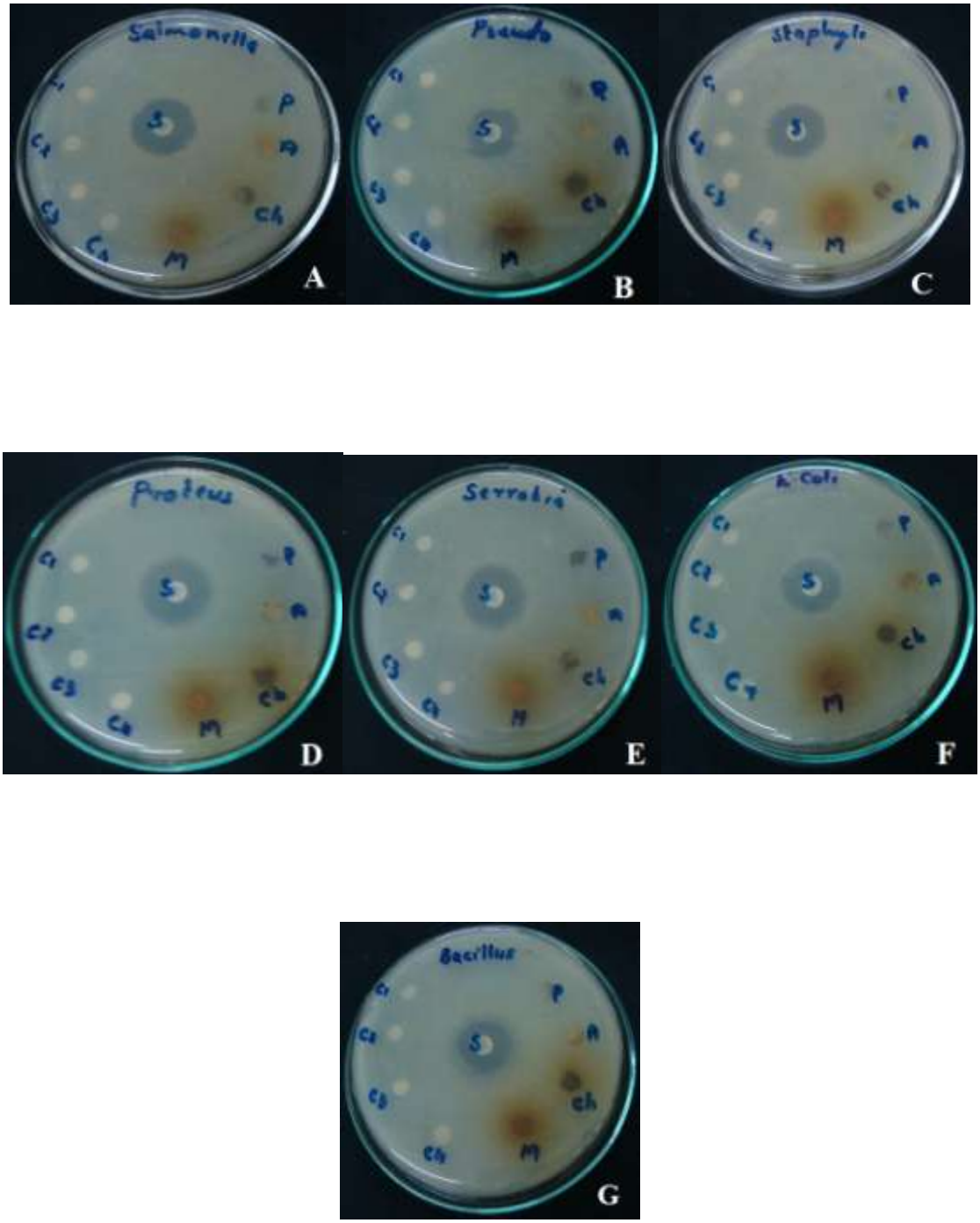

Fig: 1- Antibacterial studies of *Gloriosa superba* tuber. A- *Salmonella typhi,* **B -** *Pseudomonas aeruginosa,* **C-** *Staphylococcus aureus,* **D -** *Proteus vulgaris,* **E -** *Serratia marcescens,* **F-***Escherichia coli,* **G -** *Bacillus species*

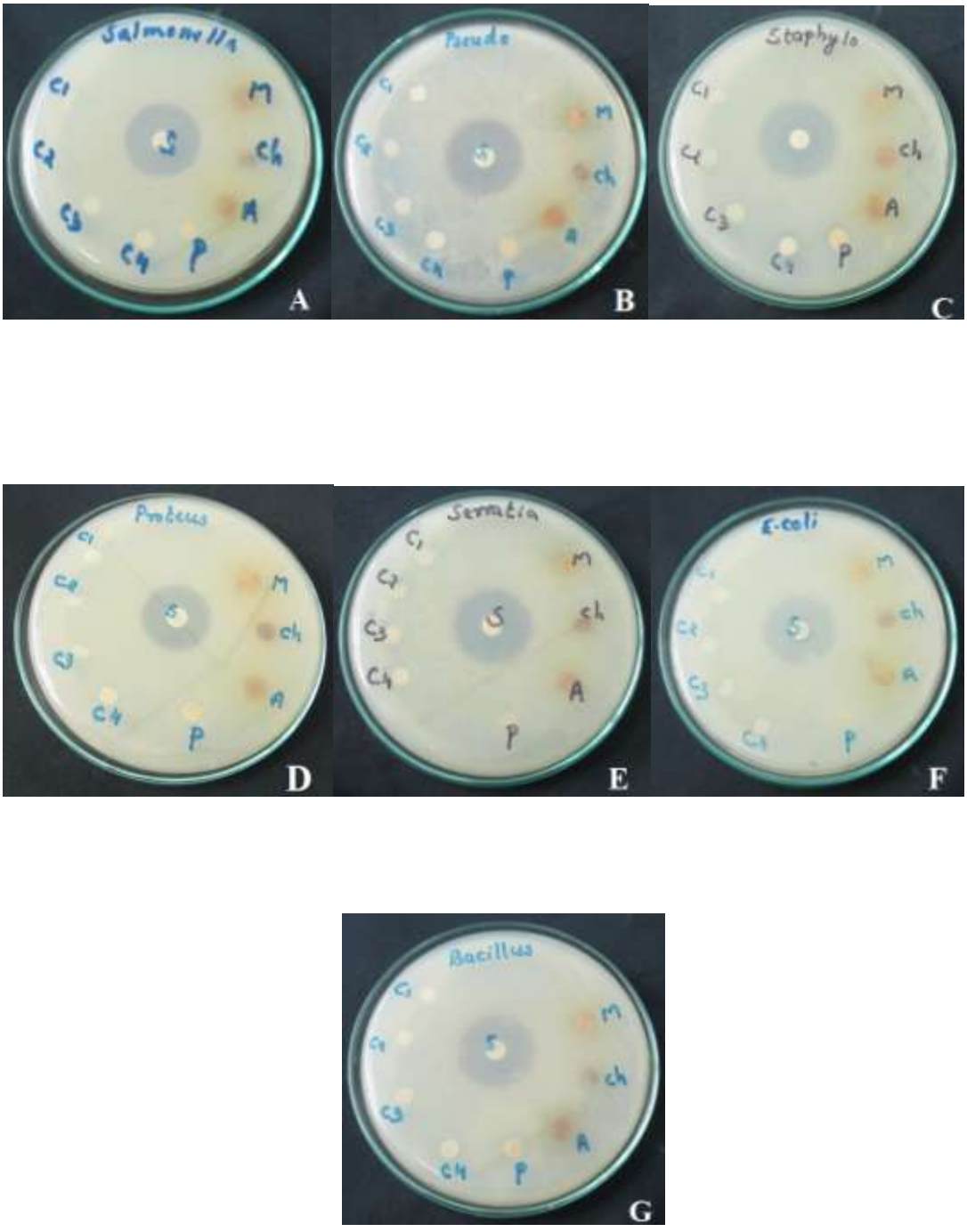

Fig: 2- Antibacterial studies of *Gloriosa superba* seed. A- *Salmonella typhi*, **B -** *Pseudomonas aeruginosa*, **C-** *Staphylococcus aureus*, **D -** *Proteus vulgaris*, **E -** *Serratia marcescens*, **F-***Escherichia coli*, **G -** *Bacillus species*

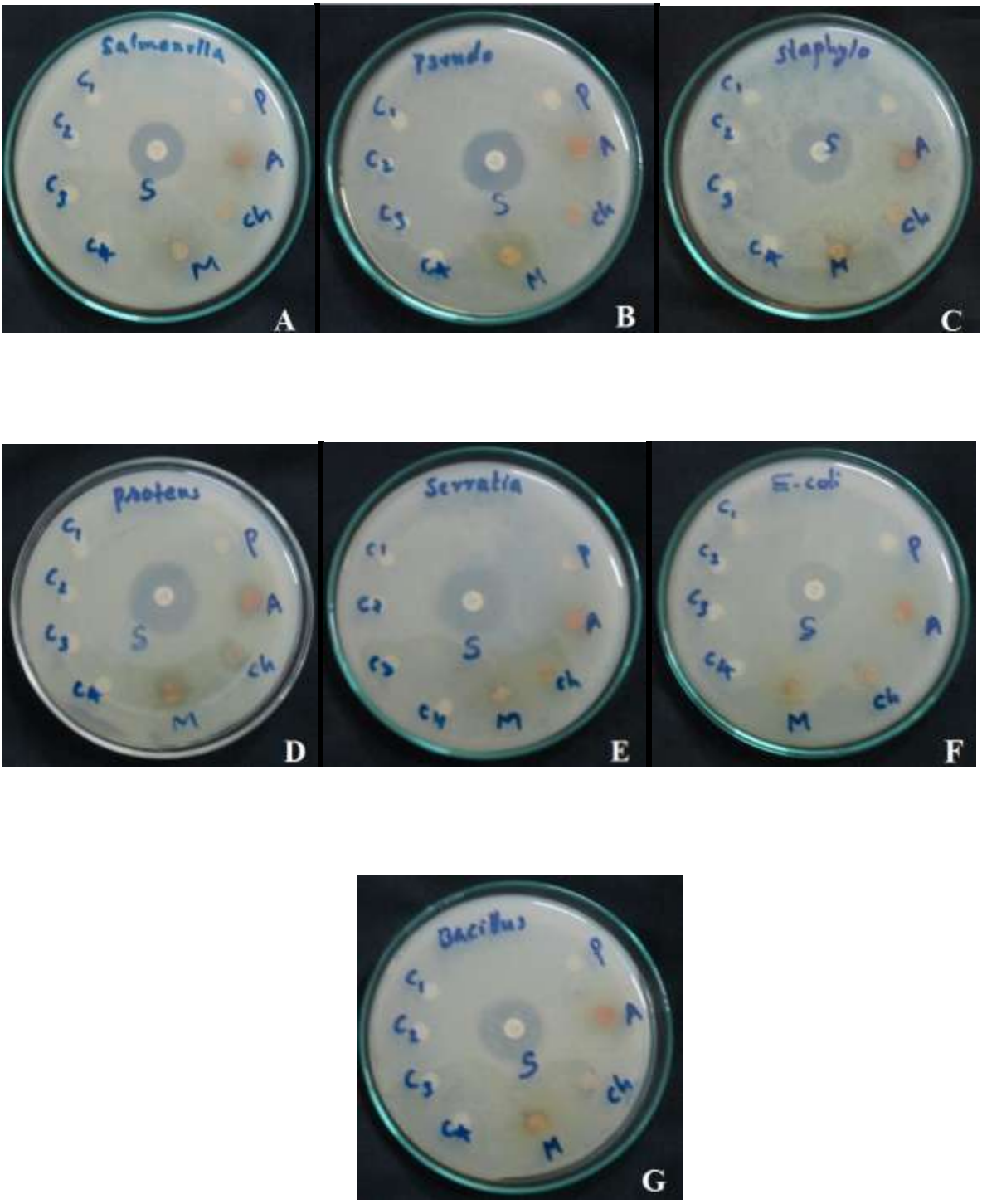

Fig: 3- Antibacterial studies of *Gloriosa superba* flower. A- *Salmonella typhi*, **B -** *Pseudomonas aeruginosa*, **C-** *Staphylococcus aureus*, **D -** *Proteus vulgaris*, **E -** *Serratia marcescens*, **F-***Escherichia coli*, **G -** *Bacillus species*

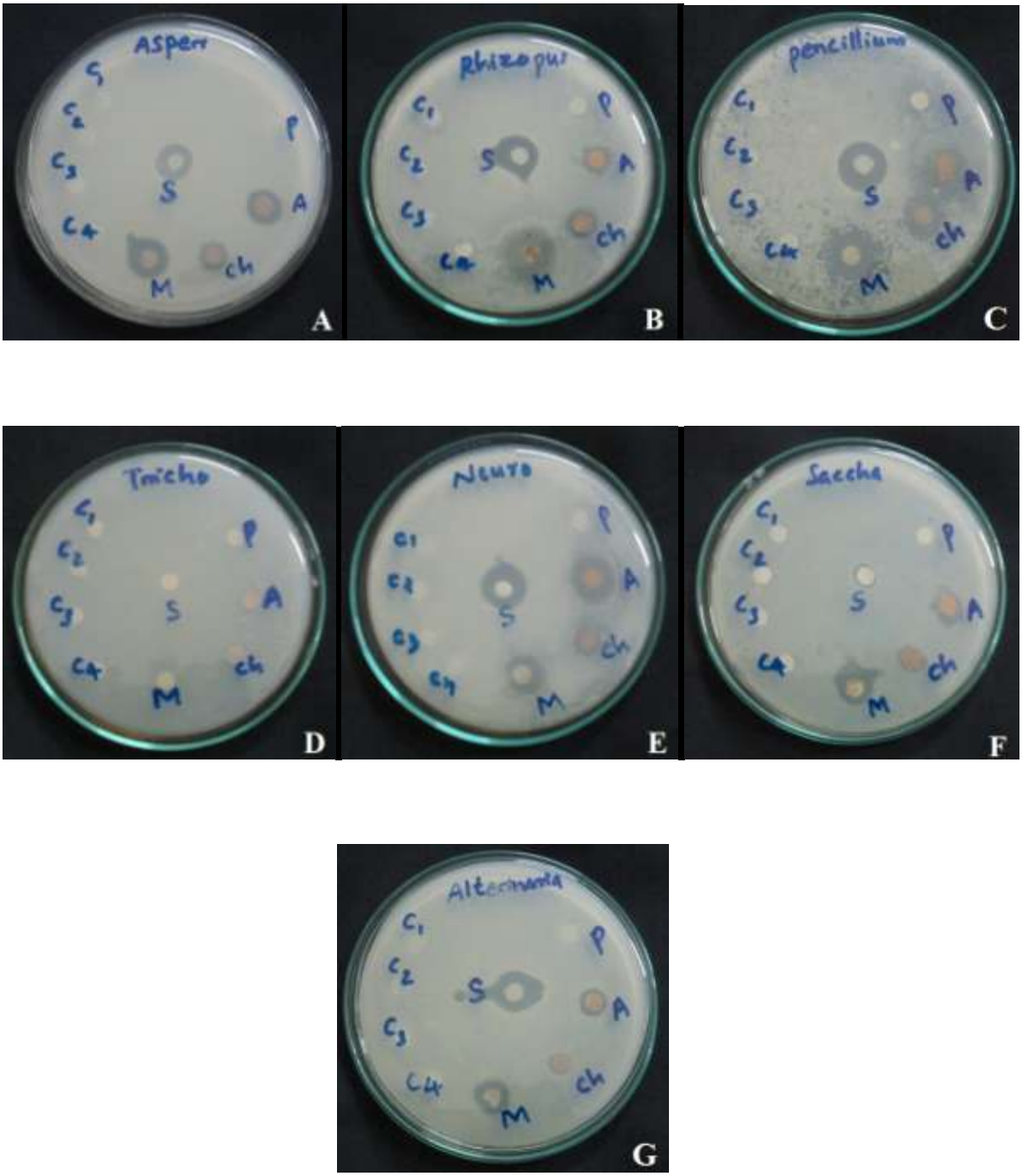

Fig: 4- Antifungal studies of *Gloriosa superba* tuber. A- *Salmonella typhi*, **B -** *Pseudomonas aeruginosa*, **C-** *Staphylococcus aureus*, **D -** *Proteus vulgaris*, **E -** *Serratia marcescens*, **F-***Escherichia coli*, **G -** *Bacillus species*

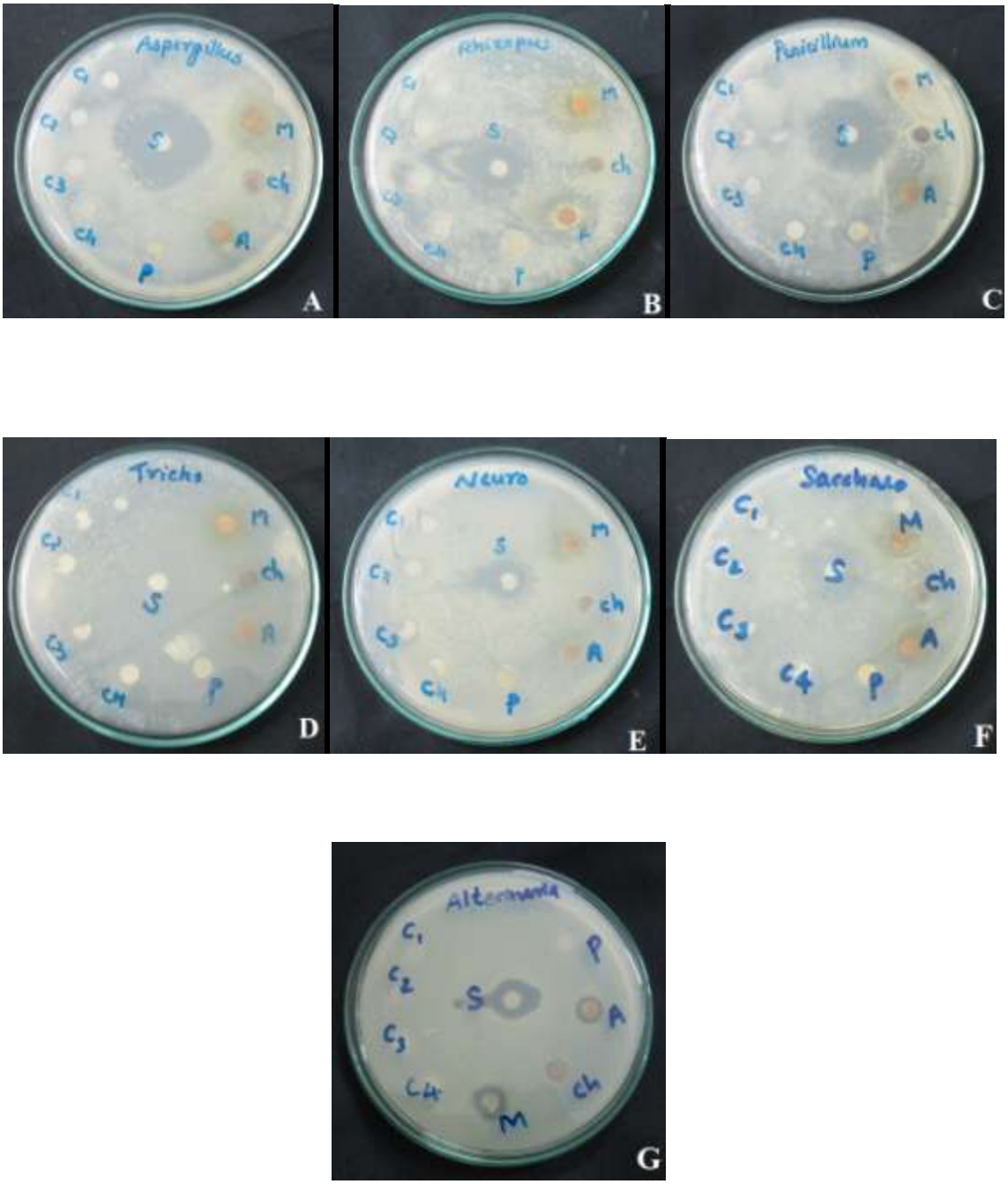

Fig: 5- Antifungal studies of *Gloriosa superba* seed. A- *Salmonella typhi,* **B -** *Pseudomonas aeruginosa,* **C-** *Staphylococcus aureus,* **D -** *Proteus vulgaris,* **E -** *Serratia marcescens,* **F-***Escherichia coli,* **G -** *Bacillus species*

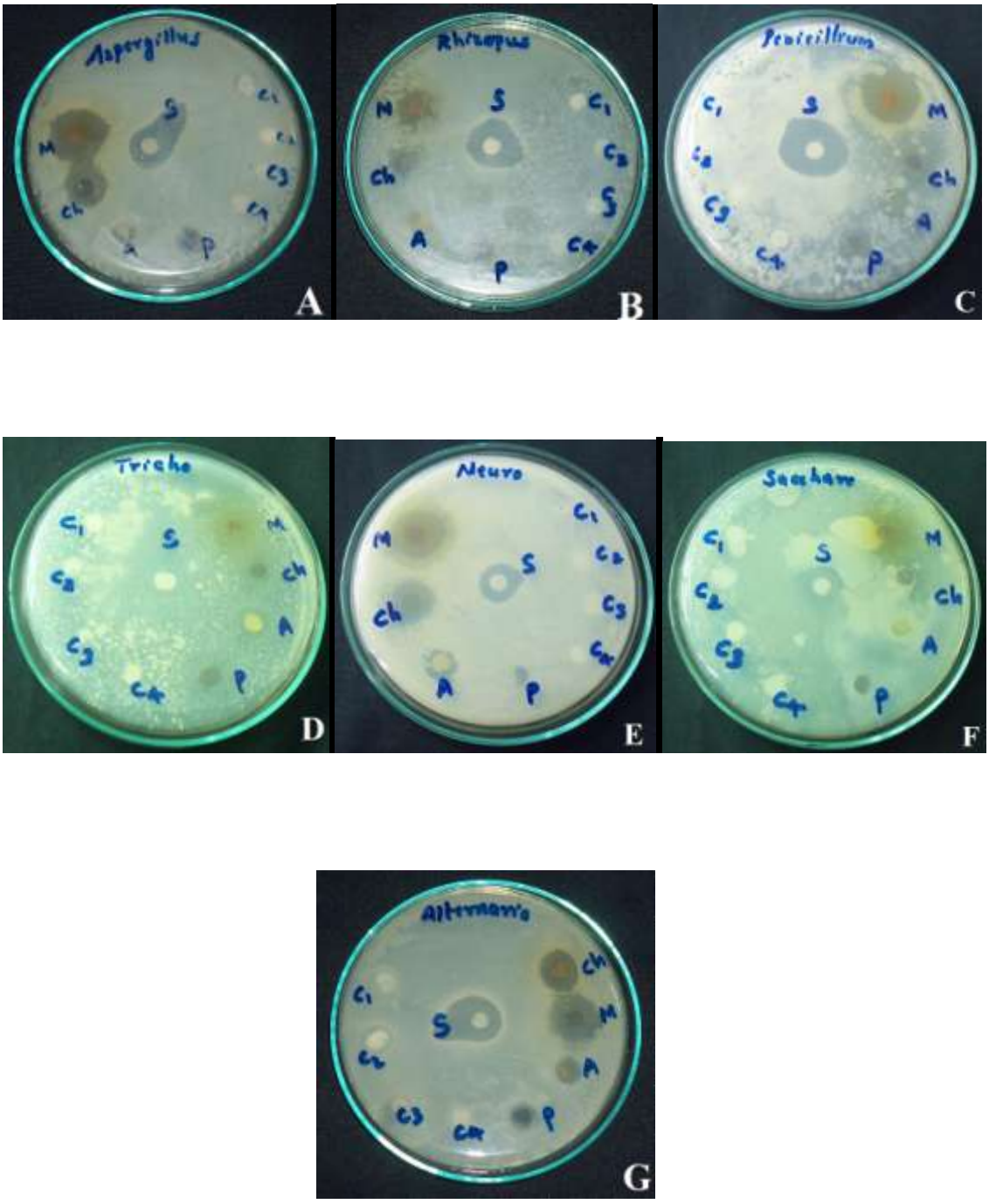

Fig: 6- Antifungal studies of *Gloriosa superba* flower. A- *Salmonella typhi*, **B -** *Pseudomonas aeruginosa*, **C-** *Staphylococcus aureus*, **D -** *Proteus vulgaris*, **E -** *Serratia marcescens*, **F-***Escherichia coli*, **G -** *Bacillus species*

CONCLUSION

It could be concluded that the extracts prepared from the tuber, seed and flower of *Gloriosa superba* are a source of different bioactive molecules which may act against different microbes. This study could be the initial step for the isolation and identification of new antimicrobial compounds for the production of a potent drug source.

ACKNOWLEDGEMENT

Authors are thankful to the Department of Botany, University College, Thiruvananthapuram**.**

REFERENCES

Abhishek M, Satish K V, Santosh K S, Deepika M, Prasad G B K S and Dua V K (2011). Investigation of anti-inflammatory properties of *Swertia chirayta* and *Gloriosa superba*. *Recent research in science and technology* **3**(3) 40-43.

Ashok K K, Shagufta K, Sujata G and Amarjeet B (2011). *In vitro* shoot initiation from apical shoot buds and meristems of *Gloriosa superba* L. - an endangered medicinal herb of high commercial value. *Researcher* **3**(11) 36- 45.

Elgayyar M, Draughon F A, Golden D A and Mount J R (2001). Antimicrobial activity of essential oils from plant against selected pathogenic and saprophytic microorganism. *Journal of Food Prot*ection **64**(7) 1019-1024.

Haroon K, Murad A K, Tahira M and Muhammad I C (2008). Antimicrobial activities of *Gloriosa superba extracts, Journal of enzyme inhibition and medicinal chemistry* **22**(6) 722-725.

Haroon R B and Nagarajan N (2011). Antibacterial potential of Glory lily, *Gloriosa superba L. International Research Journal of Pharmacy* **2**(3) 139-142.

Joshi P (1993). Tribal remedies against snake bites and scorpion stings in Rajasthan. *Glimpses Plant. Researcher* **10** 23-25.

Kamna B and Anirudha R (2012). Antimicrobial efficacy of an endemic plant species - *Gloriosa superba* L. *International Journal of Pharmacy and Biological Science* **3**(4) 353-359.

Khan H, Khan M A, Mahmood T and Choudhary M I (2008). Antimicrobial activities of *Gloriosa superba* L. extracts. *Journal of enzyme inhibition and medicinal chemistry* **23**(6) 855- 859.

Ravindra A and Mahendra K R (2009). Review: Current advances in *Gloriosa superba L. Biodiversitas* **10**(4) 210-214.

Rehana B and Nagarajan N (2012). Phytochemical screening for active compounds in *Gloriosa superba* leaves and tubers. *Journal of Pharmacognosy and Phytochemical Research* **4**(1) 17-20.

Senthilkumar M (2013). Phytochemical screening and antibacterial activity of *Gloriosa superba* L. *Journal of Pharmacognosy and Phytochemical Research* **5**(1) 31-36.

Shanmugham H, Rathinam R, Chinnathambi A and Venkatesan T (2009). Antimicrobial and mutagenic properties of the root tubers of *Gloriosa superba* L. *Pakistan Journal of Botany* **41**(1) 293-299.