ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (499-508), Month: July - September 2018, Available at: www.researchpublish.com

Antimicrobial studies on seed extracts of *Gloriosa superba* L

[1]Babu Rao Karasala, [2]Rajanikanth Garapati, [3]Rajesh Govindan,
[4]Mani Rathinasabapathi Arulmozhi, [5*]Ramesh Narayanaperumal

[1,2,3,4,] Research Scholar, [5]Assistant Professor
[1, 2] Department of Biotechnology, JJ College of Arts and Science, Pudukkottai, Tamil Nadu, India
[3, 4, 5] PG & Research Department of Botany, Government Arts College for Men, Krishnagiri, Tamil Nadu, India

Abstract: ***Gloriosa superba*** **L. belongs to the family Colchicaceae. Locally known as Sengantha malar in Tamil, Glory Lily in English. It is a perennial climbing plant having a V-shaped tuber that is found all over India. The plant traditionally used for the treatment of several human diseases like cancer, piles, and purgative. But, it also initiated disorders and mortalities to humans due to purposeful and accidental poisoning. The seeds highly valued in the world market as sources of colchicine, a chemical that has used in a remedy against gout, skin related diseases and anticancer. The antibacterial activity of aqueous, methanol, chloroform and hexane extracts of the seed of *G. superba* determined by agar well diffusion method. The aqueous, methanol, chloroform and hexane seed extracts ere screened for their antimicrobial efficiency against gram-negative bacteria. Among the different solvent extracts, the antimicrobial activity was high in the methanol, extract followed by chloroform, hexane extracts. The antimicrobial activity was much less in the cold aqueous extract than boiled extract. The efficacy of antimicrobial activity depends on the nature and volume of active principles present in the tested extracts. The antimicrobial effect of the crude seed extracts is better than standard antibiotic drugs of ampicillin and tetracycline. The tetracycline showed a better inhibitory effect against *E. cloacae, E. coli, K. aerogenes, K. pneumonia, S. typhimurium, S. maltophilia, B. megaterium, B. subtilis* and gave low inhibitory effect in *P. aeruginosa, P. maltophilia, P. oleovorans* than seed crude extract of *G. superba*.**

Keywords: ***Gloriosa superba*****, V- shape tuber, Seed, Antimicrobial activity, Ampicillin.**

I. INTRODUCTION

Herbal drugs are great demand in developed counties of the world for primary health care because of their efficiency, safety, and lesser side effects. India despite its rich traditional information, the heritage of herbal medicines and mega biodiversity has a dismal share of the globalmarket due to the export of crude plant extracts and drugs. The plants have medicinal value including antimicrobial properties. The microbial infections are greatest serious global health problems of the present century [1] particularly, bacterial diseases account for high proportion of health problems in developing countries. Numerous synthetic antibiotics are regularly used to control the bacterial disease. Due to unselective use of synthetic antibiotics, bacteria have developed resistance against many antibiotics and as a result, the enormous clinical problem in the treatment of infectious diseases [2]. The beginning of antimicrobial resistance pathogens now to treats the innovation of potent antimicrobial agents. Plant-based medicines considered safe and alternatives to synthetic drugs [3]

Gloriosa superba L. belongs to the family Colchicaceae. Locally known as Kannuvalli Kodi, Sengantha malar in Tamil, Agnisikha, Agnimukhi, in Sanskrit, Climbing lily, Glory Lily in English. *Gloriosa superba* occurs in grassland, semi-shade, coastal dunes, coastal woodlands, forest in the Botswana, Eastern Cape, India, Limpopo, Namibia, southeastern Asia, Swaziland, tropical Africa, and Zimbabwe. It is a perennial climbing plant having a V-shaped or finger-like tuber that is found all over India. The plant traditionally used for the treatment of several human diseases like cancer, gout, piles, scrofula [4] and act as antipyretic, anti-abortive and purgative [5]. But, it also initiated disorders and mortalities to humans and animals due to purposeful and accidental poisoning. The plant presently cultivated all over the world as an ornamental plant and medicinal herb [6].

The seeds highly valued in the world market as sources of colchicine, a chemical that has used in a remedy against gout, a disease was initiated by deposits of uric acid in the joins [7], skin related diseases [8], relieving rheumatic pain and muscle relaxant [9]and anticancer [10]. Few compounds are isolated from the seed such as Xanthones, Colchicines [11], Pentadecanoic acid, Hexadecanoic acid, ethyl ester, [8], 3-demethylthiocolchicine, tetraacetatecolchicoside, *colchicoside and thiocolchicoside* [12], 3-O-demethylcolchicine-3-Oalpha- D-glucopyranoside [13]. Based on the above-said information, the present study is to attempt the antibacterial efficiency of the seed of *G. superba*.

II. MATERIALS AND METHODS

The seed of *G. superba* purchased from Sivakasi, Virudhunagar District, Tamil Nadu, India. The plants propagated through seeds in polybags containing a mixture of red soil and farmyard manure in the ratio of 2:1 at botanical gardens, J.J. College of Arts and Science, Pudukkottai, Tamilnadu, India.

2.1 Successive extractive value:

The seeds are allowed to dry in shade and coarse powder. The coarse powder was successive extraction with hexane, chloroform, and methanol in the order of increasing polarity using Soxhlet apparatus [14].

2.2 Determination of Antimicrobial Activity:

The antibacterial activity of aqueous, methanol, chloroform and hexane extracts of the seed of *G. superba* determined by agar well diffusion method [15]. The plant material of 20 g was weighed, chopped and divided into two portions. Each portion was crushed by grinding in a mortar and transferred to a suitable glass bottle, and a 50 ml of distilled water added. One glass bottle with extracts was boiled (100 ºC) for 20 min, and the second was mechanically shaken (200 rpm) in a cold condition for two hours. The extracts were filtered off using cheesecloth, followed by 0.45µ filter paper and transferred into a sterile closed container. The crude extract considered as 100 % extract. By adding sterile distilled water, 50 % of the extract was prepared [16]. The various concentrations of 20, 10, 5, and 2.5 mg/ml seed extracts of hexane, chloroform, and methanol were prepared for antimicrobial activity.

2.3 Test Bacteria:

Thirteen human pathogenic bacterial strains *Aeromonas veronii, Bacillus megaterium, Bacillus subtilis, Enterobacter cloacae, Escherichia coli, Klebsiella aerogenes, Klebsiella pneumonia, Pseudomonas aeruginosa, Pseudomonas maltophilia, Pseudomonas oleovorans, Salmonella typhimurium, Staphylococcus aureus, Stenotrophomonas maltophilia* obtained from National Collection of Industrial Microorganisms, National Chemical Laboratory, Pune, and used for antimicrobial studies. The strains were kept at 4°C on agar slant and subcultured at 37°C for 24 hrs in nutrient agar for bacteria before *in vitro* susceptibility tests.

Agar well diffusion method [15] was adopted to determine the antimicrobial activity. Nutrient agar (NA) plates were swabbed (Sterile cotton swabs) with 8 hours old- broth culture of respective bacteria. Two wells (8 mm diameter) were made in each of these plates using sterile cork borer, and about 0.3 ml of 100 % and 50 % aqueous extract and different concentration of plant solvent extracts were added using sterilized dropping pipettes into the wells and allowed to diffuse at room temperature for 2 hours. The Petri plates were incubated for 18-24 hours at 37ºC for bacterial pathogens, and individual proper control of solvent plant extracts also maintained. The diameter of the inhibition zones recorded. Triplicate was maintained, and the experiment repeated thrice, and the average values recorded for antimicrobial activity.

2.4 Statistical analysis:

All experiments carried out thrice, and the data values recorded. The data were processed statistically using standard deviation and also calculated standard error. The processed data were tabulated in tables and represented in the form of a bar diagram of the graph

III. RESULTS AND DISCUSSION

3.1 Antimicrobial activity:

The aqueous, methanol, chloroform and hexane seed extracts of an ethnomedicines G. *superba* screened for their antimicrobial efficiency against gram-negative bacteria of *A. veronii, E. cloacae, E. coli, K. aerogenes, K. pneumonia, P. aeruginosa, P. maltophilia, P. oleovorans, S. typhimurium, S. maltophilia* and gram-positive bacteria of *B. megaterium, B.*

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (499-508), Month: July - September 2018, Available at: www.researchpublish.com

subtilis, *S. aureus.* Among the different solvent extracts, the antimicrobial activity was high in the methanol extract followed by chloroform and hexane extracts. The antimicrobial activity was much less in the cold aqueous extract than boiled extract. The efficacy of antimicrobial activity depends on the nature and volume of active principles present in the tested extracts. The aqueous extract showed activity in both gram-positive and gram-negative bacteria except *P. maltophilia* and *B. subtilis* (Table – 1, Graph - 1). The methanol, chloroform, and hexane extracts inhibited both gram-negative and gram-positive bacteria (Table - 2,3,4 Graph - 2,3,4).

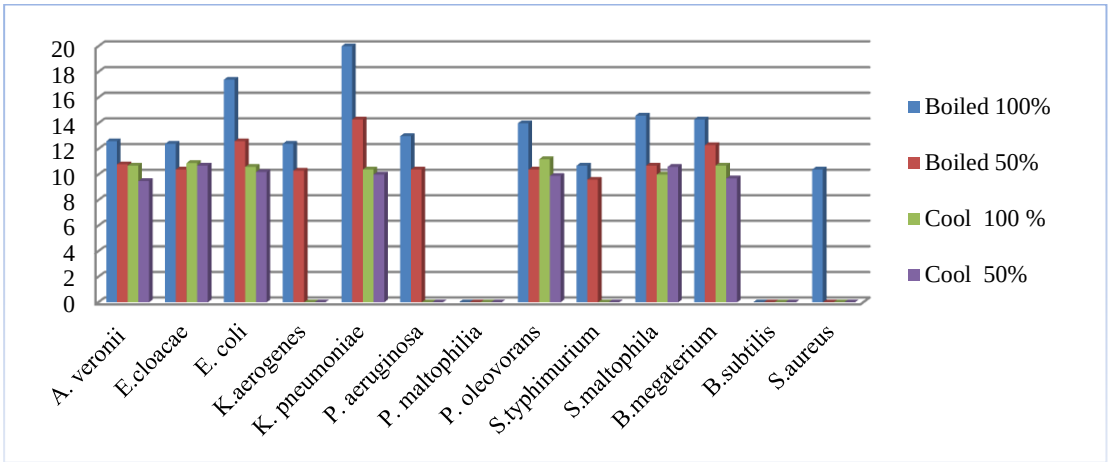
3.2 Antimicrobial activity of aqueous extract:

The cold aqueous extract showed maximum inhibition zone of 11.2±0.7 mm in 100 % against *P. oleovorans* and 10.7±0.2 mm in 50 % against *E. cloacae*. Minimum inhibition zone of 10.0±0.8 mm in 100 % against *S. maltophilia* and 9.5±0.7 mm in 50 % concentrationwere observed in *A. veronii* (Table – 1). There is no zone of inhibition was observed against *K. aerogenes*, *P. aeruginosa*, *P. maltophilia*, *S. typhimurium*, *B. subtilis*, and *S. aureus.*

Table 1: Antimicrobial activity of methanol extract of *G. superba* seed

Organism	NCIM Acc. No	Boiled Extracted		Cooled Extracted	
		100%	50%	100%	50%
A. veronii	5621	12.6±0.9	10.8±0.6	10.7±0.2	9.5±0.7*
E. cloacae	2164	12.4±0.6	10.4±0.2	10.9±0.7	10.7±0.2
E. coli	2068	17.4±0.3	12.6±0.3	10.6±0.2	10.2±0.6
K. aerogenes	2239	12.4±0.2	10.3±0.7	-	-
K. pneumoniae	2707	20.0±0.4	14.3±0.2	10.4±0.8	10.0±0.7
P. aeruginosa	3037	13.0±0.9	10.4±0.5	-	-
P. maltophilia	2866	-	-	-	-
P. oleovorans	2867	14.0±0.6	10.4±0.9	11.2±0.7	9.9±0.3
S. typhimurium	2501	10.7±0.2	9.6±0.5	-	-
S. maltophilia	5625	14.6±0.9	10.7±0.4	10.0±0.8	10.6±0.2
B. megaterium	2052	14.3±0.3	12.3±0.8	10.7±0.2	9.7±0.5
B. subtilis	2920	-	-	-	-
S. aureus	2079	10.4±0.7	-	-	-

* Zone of inhibition in mm



Graph 1: Antimicrobial activity of methanol extract of *G. superba* seed

The boiled aqueous extract showed maximum inhibition zone of 20.0±0.4 mm in 100% and 14.3±0.2 mm in 50 % concentrations against *K. pneumoniae*. Minimum inhibition zone of 10.4±0.7 mm in 100 % against *S. aureus* and 9.6±0.5 mm in 50 % concentration observed in *S. typhimurium* (Table - 1, Graph - 1). The antimicrobial activity of the boiled aqueous extract showed better antimicrobial activity in gram-negative bacteria than positive bacteria. Similarly, the cold aqueous extract also shows inhibitory in gram-negative bacteria. The cold aqueous seed extract has shown low inhibitory activity in *K. pneumonia*, but better activity on boiled extract. This study revealed that the high percentage of active principle should be dissolved in hot water extract than cold.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (499-508), Month: July - September 2018, Available at: www.researchpublish.com

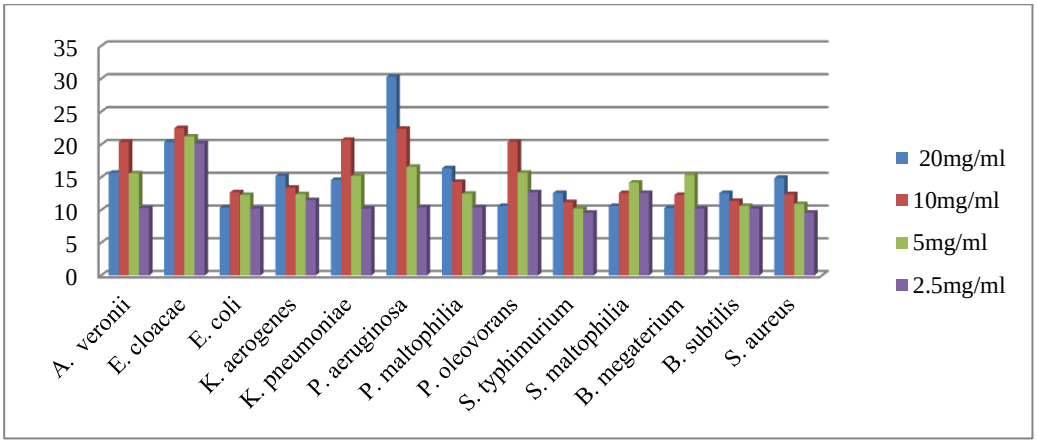
3.3 Antimicrobial activity of methanol extract:

The antibacterial activity of methanol extract (Table - 2) showed better inhibition zone of 30.3±0.7mm in 20 mg/ml, 22.4±1.3 in 10 mg/ml against *P. aeruginosa*; 21.2±0.5 mm in 5 mg/ml and 20.4±0.3 mm in 2.5 mg/ml against *E. cloacae* (Table – 2,Graph - 2).

Table 2: Antimicrobial activity of methanol extract of *G. superba* seed

Organism	NCIM Acc. No	Methanol extract mg/ml			
		20	10	5	2.5
A. veronii	5621	15.7±0.2	20.4±1.3	15.6±0.8	10.3±0.2*
E. cloacae	2164	20.4±0.6	22.5±0.2	21.2±0.5	20.2±0.3
E. coli	2068	10.4±0.7	12.7±0.2	12.3±0.2	10.2±0.3
K. aerogenes	2239	15.2±0.3	13.4±0.2	12.4±0.7	11.5±0.3
K. pneumoniae	2707	14.6±0.8	20.7±0.2	15.2±1.3	10.2±0.7
P. aeruginosa	3037	30.3±0.7	22.4±1.3	16.6±0.8	10.4±0.7
P. maltophilia	2866	16.4±0.6	14.3±0.2	12.5±0.2	10.4±0.2
P. oleovorans	2867	10.6±0.7	20.4±0.3	15.7±0.8	12.7±0.9
S. typhimurium	2501	12.6±0.3	11.2±0.6	10.2±0.5	9.6±0.3
S. maltophilia	5625	10.6±0.3	12.6±1.2	14.2±1.6	12.6±1.0
B. megaterium	2052	10.3±0.9	12.3±0.5	15.4±0.3	10.2±0.8
B. subtilis	2920	12.6±0.9	11.4±0.7	10.6±0.2	10.2±0.5
S. aureus	2079	14.9±0.4	12.4±0.2	10.9±0.3	9.6±0.5

* Zone of inhibition in mm



Graph 2: Antimicrobial activity of methanol extract of *G. superba* seed

The minimum inhibitory zone 9.6±0.3 mm was seen in 2.5 mg/ml and 10.2±0.5 mm in 5 mg/l against *S. typhimurium;* 10.3±0.9 mm in 20 mg/ml against *B. megaterium*, 11.2±0.6 mm in 10 mg/ml against *S. typhimurium* (Table – 2, Graph - 2). The methanol seed extracts *G. superba* showed better activity in gram-negative bacteria than gram-positive.

3.4 Antimicrobial activity of chloroform extract:

The antibacterial activity of chloroform extract (Table - 3) showed better inhibition zone of 22.6±0.4 mm in 20 mg/ml, 21.4±0.3 mm in 10 mg/ml, 17.6±0.2 mm in 5 mg/ml, 16.2±0.4 mm in 2.5 mg/ml against *E. cloacae* (Table – 3, Graph - 3).

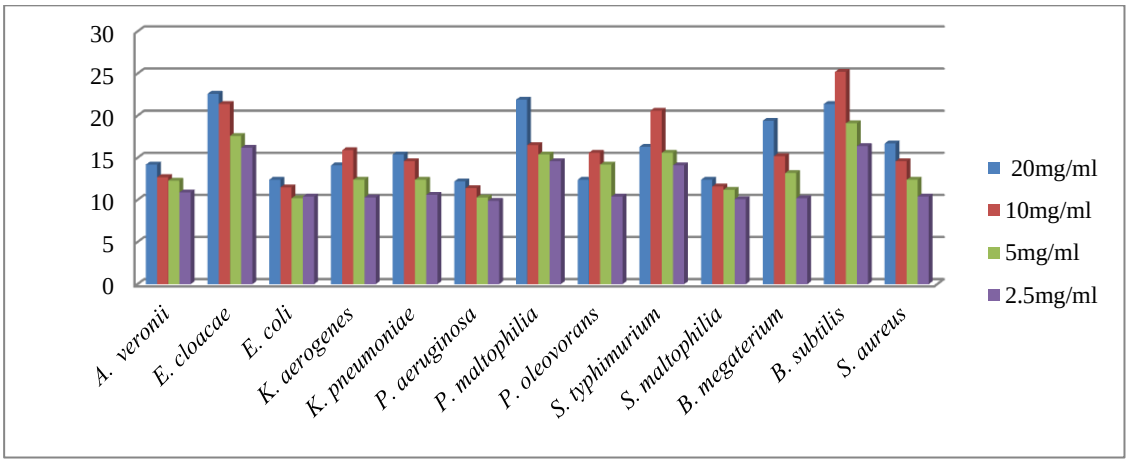
Table 3: Antimicrobial activity of chloroform of *G. superba* seed

Organism	NCIM Acc. No	Chloroform extract mg/ml			
		20	10	5	2.5
A. veronii	5621	14.2±0.4	12.7±0.3	12.3±0.8	10.9 ±0.3*

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (499-508), Month: July - September 2018, Available at: www.researchpublish.com

E. cloacae	2164	22.6±0.4	21.4±0.3	17.6±0.2	16.2±0.4
E. coli	2068	12.4±0.3	11.5±0.7	10.2±0.2	10.4±0.9
K. aerogenes	2239	14.1±0.3	15.9±0.2	12.4±0.7	10.3±0.3
K. pneumoniae	2707	15.4±0.2	14.6±1.3	12.4±0.3	10.6±0.6
P. aeruginosa	3037	12.2±0.6	11.4±0.7	10.3±0.9	9.9±1.1
P. maltophilia	2866	21.9±0.2	16.5±0.7	15.4±1.2	14.6±0.9
P. oleovorans	2867	12.4±0.2	15.6±0.8	14.2±0.3	10.4±0.2
S. typhimurium	2501	16.3±0.3	20.6±0.9	15.6±0.7	14.1±0.3
S. maltophilia	5625	12.4±1.2	11.6±0.3	11.2±0.7	10.1±1.3
B. megaterium	2052	10.3±0.7	12.3±0.5	15.4±0.3	10.2±0.8
B. subtilis	2920	12.6±0.9	11.4±0.3	10.6±0.2	10.2±0.5
S. aureus	2079	16.7±0.2	14.6±0.2	12.4±0.1	10.4±0.3

* Zone of inhibition in mm



Graph 3: Antimicrobial activity of chloroform of *G. superba* seed

The low inhibitory zone 9.9±1.1 mm showed in 2.5 mg/ml concentration against *P. aeruginosa*, 10.2±0.2 mm in 5 mg/l against *E. coli*, 10.3±0.7 mm in 20 mg/ml against *B. megaterium*; 11.4±0.3 mm in 10 mg/ml against *B. subtilis*. The antimicrobial activity of the chloroform extracts expresses better activity in low concentration compare to high concentration against *S. typhimurium*, *S. aureus*, *K. aerogenes*. The dilution factor mainly contributes to the efficiency of antimicrobial activity in chloroform extract.

3.5 Antimicrobial activity of hexane extract:

The antibacterial activity of hexane extract (Table - 4) showed better inhibition zone of 30.5±0.8 mm in 10 mg/ml and 25.2±0.3 mm in 5 mg/ml against *A. veronii*; 20.7±0.7 mm in 20 mg/ml against *K. aerogenes*; 15.9±0.7mm in 2.5 mg/ml against *A. veronii* (Table – 4, Graph - 4). The low inhibitory zone 9.4±0.6 mm showed in 2.5 mg/ml and 10.2±0.1 mm in 5 mg/ml concentrations against *P. maltophilia*, 11.4±1.6 mm in 10 mg/ml and 12.2±0.1 mm in 20 mg/ml against *K. pneumonia*.

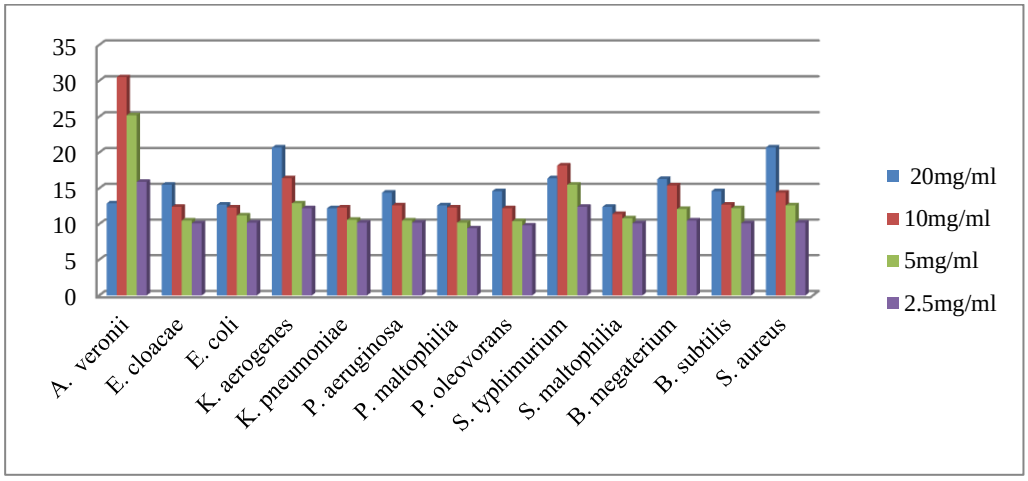
Table 4: Antimicrobial activity of hexane extract of *G. superba* seed

Organism	NCIM Acc. No	Hexane extract mg/ml			
		20	10	5	2.5
A. veronii	5621	12.9±0.3	30.5±0.8	25.2±0.3	15.9±0.7*
E. cloacae	2164	15.5±0.4	12.4±0.6	10.5±0.2	10.1±0.2
E. coli	2068	12.7±0.5	12.2±0.5	11.2±0.6	10.2±0.3
K. aerogenes	2239	20.7±0.7	16.4±1.3	12.9±0.5	12.2±0.4
K. pneumoniae	2707	12.2±0.1	12.3±0.2	10.6±0.2	10.2±0.4
P. aeruginosa	3037	14.4±0.3	12.6±0.2	10.5±0.3	10.2±0.2

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (499-508), Month: July - September 2018, Available at: www.researchpublish.com

P. maltophilia	2866	12.6±0.9	12.3±0.2	10.2±0.1	9.4±0.6
P. oleovorans	2867	14.6±0.3	12.2±0.9	10.4±0.9	9.8±0.7
S. typhimurium	2501	16.4±0.3	18.2±0.1	15.5±0.2	12.4±0.7
S. maltophilia	5625	12.4±0.7	11.4±1.6	10.8±0.6	10.1±0.4
B. megaterium	2052	16.3±0.8	15.4 ±0.6	12.1±0.3	10.5±0.9
B. subtilis	2920	14.6±0.2	12.7±0.6	12.2±0.5	10.1±0.3
S. aureus	2079	20.7±0.2	14.4±0.7	12.6±0.3	10.2±0.7

* Zone of inhibition in mm



Graph 4: Antimicrobial activity of hexane extract of *G. superba* seed

The antimicrobial activity of the hexane extracts expresses better activity against gram-positive organism than the negative organism. The hexane extract showed maximum activity in *A. veronii* among the tested organism (Table – 4, Graph – 4).

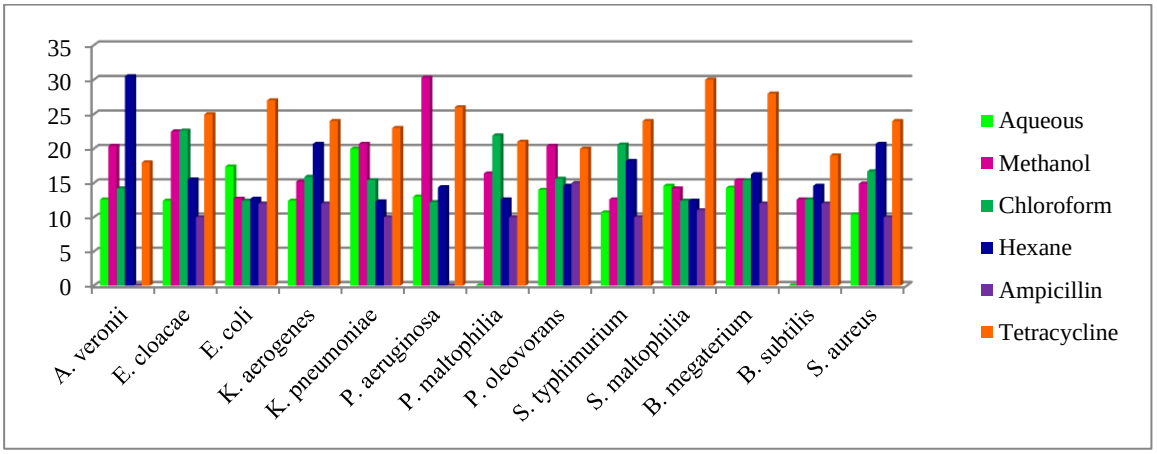
Aqueous extracts predominantly inhibited the gram-negative bacteria of *E. coli*, and *S. maltophilia* growth compares to all other extracts. It denoted that the aqueous extract of seed having an active principle for controlling the *E. coli* and *S. maltophilia* infection compare to other extracts (Table – 5). The methanol extracts shown better inhibitory activity *against K. pneumonia*, *P. aeruginosa and P. oleovorans* among the tested bacteria. Based on this study, the methanol seed extract of *G. superba* has active secondary metabolites including alkaloids may be controlled by these organisms efficiently than other organisms.

Table 5: Antimicrobial activity of *G. superba* seed extracts vs. standard antibiotics

Organism	NCIM Acc. No	Aqueous	Methanol	Chloroform	Hexane	Ampicillin 10 mcg	Tetracycline 30 mcg
A. veronii	5621	12.6±0.9	20.4±1.3	14.2±0.4	30.5±0.8	-	18±0.7
E. cloacae	2164	12.4±0.6	22.5±0.2	22.6±0.4	15.5±0.4	10±0.6	25±0.4
E. coli	2068	17.4±0.3	12.7±0.2	12.4±0.3	12.7±0.5	12±0.7	27±0.2
K. aerogenes	2239	12.4±0.2	15.2±0.3	15.9±0.2	20.7±0.7	12±0.2	24±0.8
K. pneumoniae	2707	20.0±0.4	20.7±0.2	15.4±0.2	12.3±0.2	10±0.3	23±0.5
P. aeruginosa	3037	13.0±0.9	30.3±0.7	12.2±0.6	14.4±0.3	-	26±0.3
P. maltophilia	2866	-	16.4±0.6	21.9±0.2	12.6±0.9	10±0.9	21±0.7
P. oleovorans	2867	14.0±0.6	20.4±0.3	15.6±0.8	14.6±0.3	15±0.4	20±0.9
S. typhimurium	2501	10.7±0.2	12.6±0.3	20.6±0.9	18.2±0.1	10±0.6	24±0.2
S. maltophilia	5625	14.6±0.9	14.2±1.6	12.4±1.2	12.4±0.7	11±0.7	30±0.6
B. megaterium	2052	14.3±0.3	15.4±0.3	15.4±0.3	16.3±0.8	12±0.8	28±0.5
B. subtilis	2920	-	12.6±0.9	12.6±0.9	14.6±0.2	12±0.5	19±0.6
S. aureus	2079	10.4±0.7	14.9±0.4	16.7±0.2	20.7±0.2	10±0.3	24±0.9

* Zone of inhibition in mm

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (499-508), Month: July - September 2018, Available at: www.researchpublish.com



Graph 5: Antimicrobial activity of *G. superba* seed extracts vs. standard antibiotics

The chloroform extracts of seed shown better antimicrobial activity against *E. cloacae, K. aerogenes, P. maltophilia and S. typhimurium* than other tested organisms. The chloroform extracts also having some active metabolite to contribute to the control of gram-negative organism than gram-positive. The gram-negative bacteria of *A. veronii, K. aerogenes* and gram positive bacteria of *B. megaterium, B. subtilis, S. aureus* sowed maximum inhibitory effect only in hexane seed extract. The phytochemicals may contribute to the activity in hexane extract against the above-said organism. The antimicrobial effect of the crude seed extracts is better than standard antibiotic drugs of ampicillin (10 mcg) and tetracycline (30 mcg). The ampicillin shown low inhibit zone against all the organism and did not show any inhibitory effect against *A. veronii* and *P. aeruginosa* (Table – 5) but the tetracycline showed better inhibitory impact against *E. cloacae, E. coli, K. aerogenes, K. pneumonia, S. typhimurium, S. maltophilia, B. megaterium, B. subtilis* and gave low inhibitory effect in *P. aeruginosa, P. maltophilia, P. oleovorans* than seed crude extract of *G. superba*.

Medicinal plants are the main sources of new drug and may initiate a substitute to the usual medicines. The aromatic and therapeutic plants are used on a large scale to fill against drug-resistant bacteria[17]. The *G. superba* is a well-known traditional system of Indian medicine for its colchicine content which is used to treat acute gout, an antidote to snake bite, arthritis, infertility, intestinal worms, laxative, skin parasites, skin problems, spines and treatment of cancer. Phytochemical studies of seed having colchicines, 3-O-demethylcolchicine-3-Oalpha- D-glucopyranoside [13], 3demethylthiocolchicine, tetraacetatecolchicoside, 3-demethylcolchicine, N-formyl deacetylcolchcine, colchicoside and thiocolchicoside [12],[18], superbine and gloriosine, lumicolchicine, 3-demethyl-N-deformyl-N-deacetylcolchicine [13].

In our study the phytochemicals of seed having less activity against *E. coli*, but this study is controversy to earlier report said that the better inhibitory zone observed against *E. coli* because of the influence of phytochemical from the tuber of *G. superba* [19],[20].The antimicrobial activity of the crude extracts influence by some factors like the binding capacity, chelation of iron, proteins of the bacterial cell membranes and the nature of the phytochemicals [21]. In this study, *S. aureus* was inhibited by the hexane extract, because it has an active antibacterial agent, but this report is controversy to earlier research said that the methanol extract of *A. paniculata* shows 75 % of the methanol extract to control the *S. aureus* [22]. Likewise, the low concentration of methanol, chloroform, and hexane seed extracts showed a better zone of inhibition than the high dose against the tested pathogens. This observation positively correlated with the earlier research report [23-27]. All the seed extracts of *G. superba* having the potential to control both gram-positive and negative organism, and the similar effect observed in *Andrographis paniculata, Begonia malabarica, Bridelia crenulata, Drynaria quercifolia, Swertia corymbosa,* [28-34].

Naturally occurring alkaloids have nitrogenous compounds that establish the basic phytochemicals of flowering plants. Alkaloids are formed as metabolic products and have been described to be accountable for pharmaceutically active [35]. Alkaloids have identified in the extracts or compounds that have been documented to possess medicinal properties and to promote health effects [36], [37]. Glycosides are non-fragrance and serve as defense mechanisms against predation by microorganisms, insects, and herbivores [38]. These compounds served as essential drugs, which help the body to fight microbial invasion [39]. Tannins have been used traditionally for protection of reddened surfaces of the mouth and treatment of catarrh, diarrhea, hemorrhoids, and wounds. Plant tannins have accepted for their pharmacological properties [40]. Given the earlier and present results, it is clear that the plant retains the antimicrobial property of the hexane, chloroform, methanol and aqueous extracts of the seeds of *G. superba*.

IV. CONCLUSION

The antimicrobial effect of the crude seed extracts is better than standard antibiotic drugs ampicillin (10 mcg) and tetracycline (30 mcg) because it contains many effective active principles and also used in traditional system of medicine to control a number of diseases. The ampicillin showed low inhibit zone against all the organism and did not show any inhibitory effect against *A. veronii* and *P. aeruginosa*. Similarly, the standard antibiotic disc tetracycline showed a better inhibitory effect against *E. cloacae, E. coli, K. aerogenes, K. pneumonia, S. typhimurium, S. maltophilia, B. megaterium, B. subtilis* and gave low inhibitory effect in *P. aeruginosa, P. maltophilia, P. oleovorans* than seed crude extract of *G. superba*. This observation denoted that seed having some selective active principle to give more antimicrobial effect than the standard antibiotic disc ampicillin, tetracycline and to be isolated the active principle and also to develop new drugs against P. *aeruginosa, P. maltophilia, P. oleovorans* infections.

ACKNOWLEDGMENTS

Authors, Sincerely thank National Collection of Industrial Microorganism, CSIR –National Chemical Laboratory, Pune, India for providing microbial culture.

REFERENCES

[1] Gomathi, S., Ambikapathy, V. and Panneerselvam, A. 2011. Antimicrobial Activity of Some Medical Plants Against Pythium debaryanum (Hesse). J. Microbiol. Biotech. Res. 1 (2): 8-13.

[2] Bansod S., and Rai, M. 2008. Antifungal Activity of Essential Oils from Indian Medicinal Plants Against Human Pathogenic Aspergillus fumigatus and A. niger. World Journal of Medical Sciences 3 (2), 81-88.

[3] Audu S.A., Ilyas M., and Kaita H.A. 2007.Phytochemical screening of the leaves of Lophira lanceolata (Ochanaceae). Life Science Journal 4(4): 75- 79.

[4] Evans, D.A., Tanis, S.P. and Hart, D.J. 1981. A convergent totalsynthesis of (and) (F) Desacetamido isocolchicine. J Am Chem Soc 103: 5813-5821.

[5] Guhabakshi, D.N, Pal, D.C and Sersuma, P. 2001. A Lexicon of Medicinal Plants in India, Vol. 1, Naya Prokash, pp. 443-45.

[6] Bunyapraphatsara, N. and van Valkenburg, J.L.C.H. 1999. Gloriosa superba L. Pp. 289-292 in: de Padua, L.S., Bunyapraphatsara, N. and Lemmens, R.H.M.J. (eds.), Plant resources of South-East Asia: medicinal and poisonous plants 12. Backhuys Publishers, Leiden.

[7] Sivakumar, G. and Krishnamurthy, K.V. 2002. Gloriosa superba L.: a useful medicinal plant. Pp. 465-481 in: Singh, V.K., Govil, J.N., Hashmi, S. & Singh, G. (eds.), Recent progress in medicinal plants: ethnomedicine and pharmacognosy II. Sci-Tech Publishing LLC, Houston.

[8] Megala, S. And Elango, R. 2012. Bioactive Compounds Analysis Tuber and Seed of Gloriosa Superba Gc –Ms Method. International Journal of Recent Scientific Research 3(10): 871 -873.

[9] Nadkarni, A.K. 2002. Indian materia medica. Popular Prakashan limited, Mumbai.

[10] Budhiraja, A., Nepali, K., Kaul, S. and Dhar, K.L. 2012. Antimicrobial and cytotoxic activities of fungal isolates of medicinal plant Gloriosa superba. Int. J. Recent Adv. Pharm. Res. 2(1):37-45.

[11] Abhishek Mathur, Verma, S. K., Singh, S.K., Mathur, D., 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.

[12] Nautiyal, O. P. 2011. Isolation of 3-demethylcolchicine from Gloriosa superba sludge and coupling with acetobromoglucose to yield colchicoside and Thiocolchicoside. Journal of Natural Products 4: 87-93.

[13] Suri, O.P., Gupta, B.D and Suri, K.A. 2001. A new glycoside, 3-Odemethylcolchicine- 3-O-alpha-d-glucopyranoside from Gloriosa seeds. Natural Product Letters, 15: 217-219.

[14] Anonymous. 1958. british Phamacopoeia. The Pharmaceutical Press, London.

[15] Perez, C. and Paul, M. and Bazerque, P.1990. Antibiotic assay by the agar-well diffusion method. Acta Biol Medi Exp 15:113-115.

[16] Sen, S. and Nandi, P. 1951. Antibiotics from the pteridophytes. Sci Cult 16: 328-329.

[17] Tepe, B., Daferera, D., Sokmen, M., Polissiou, M. and Sokmen, A. 2004. In vitro antimicrobial and antioxidant activities of the essential oils and various extracts of Thymus eigii M. Zohary et P. H. Davis, . J Agric Food Chem 52(5):1132-7.

[18] Rahul, S., Phatak, and Laxmi Narayan Hegde. 2014. Glory Lily (Gloriosa Superba L.): An Im Por Tant Me Dic I Nal Crop– A Review. Hortflora Research Spectrum 3(3): 282-287.

[19] Hemaiswarya, S., Raja, R., Anbazhagan, C. and Thiagarajan, V. 2009. Antimicrobial and mutagenic properties of the root tubers of Gloriosa superba Linn. (Kalihari). Pak. J. Bot. 41(1): 293-299.

[20] Khan, H., Khan, M.A. and Mahmood, T. 2008. Antimicrobial activities of Gloriosa superba Linn extracts. Journal Enzyme inhibition and Medicinal Chemistry 6: 855- 859.

[21] Premkumar, P., Priya, J. and Suriyavathana, M. 2010. Evaluation of antioxidant potential of Andrographis echioides and Boerhavia diffusa, Int. J. Curr. Res 3:59-62.

[22] Mishra, P.K., Rahul Kunwar, S., Anamika, G., Adya C., Rahul, P., Shree Prakash, T. et al. 2013. Antimicrobial activity of Andrographis paniculata (Burm.f.) Wal ex Nees leaves against clinical pathogens. Journal of Pharmacy Research 7:459-462.

[23] Negi, P.S., Chauhan, A.S., Sadia, G.A., Rohinishree, Y.S. and Ramteke, R.S. 2005. Antioxidant and antibacterial activities of various seabuckthorn (Hippophaerhamnoides L.) seed extracts. Food Chem 92:119- 124

[24] Parekh, J. and Chanda, S. 2010. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. Afr J Biomed Res 10:175-181.

[25] Al-Bayati, F.A. 2008. Synergistic antibacterial activity between Thymus vulgaris and Pimpinella anisum essential oils and methanol extracts, J Ethnopharmacol 116 (3): 403-406.

[26] Kaushik, P. and Goyal, P. 2011. Evaluation of various crude extracts of Zingiber officinale rhizome for potential antibacterial activity: a study in vitro, Advance Microbiol 1:7-12.

[27] Ramesh, N., Hari, P., Rajesh, G., Babu Rao, K. and Rajanikanth, G. 2018. Antimicrobial and Phytochemical Studies of Andrographis paniculata (Burm.f.) Wall. ex Nees and Andrographis echioides (L.) Nees. Int J Life Sci Res 6(3): 334-341.

[28] Zaiden, M.R., Noor Rain, A., Badrul, A.R., Adlin, A., Norazah, A. and Zakiah, I. 2005. In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method. Tropical Biomedicine 22(2): 165–170.

[29] Humnabadkar, S.S. and Kareppa, B.M. 2012. In vitro study of antibacterial activity of Andrographis paniculata against clinically important pathogens, International Journal of Advanced Biological Research 2(4):584-586.

[30] Ramesh, N., Viswanathan, M.B., Saraswathy, A., Balakrishna, K., Brindha, P., Lakshmanaperumalsamy, P. 2001. Phytochemical and antimicrobial studies of Bridelia crenulata. Pharmaceutical Biol. 39(6): 460-464.

[31] Ramesh, N., Viswanathan, M.B., Saraswathy, A., Balakrishna, K., Brindha, P., Lakshmanaperumalsamy, P. 2001. Antibacterial activity of luteoforol from Bridelia crenulata. Fitoterapia 72:401-411.

[32] Ramesh, N., Viswanathan, M.B., Saraswathy, A., Balakrishna, K., Brindha, P., Lakshmanaperumalsamy, P. 2001. Phytochemical and antimicrobial studies on Drynaria quercifolia. Fitoterapia 72:934-936

[33] Ramesh, N., Viswanathan, M.B., Saraswathy, A., Balakrishna, K., Brindha, P., Lakshmanaperumalsamy, P. 2001. Phytochemical and antimicrobial studies on Drynaria quercifolia. Fitoterapia 72:934-936

[34] Ramesh, N., Viswanathan, M.B., Saraswathy, A., Balakrishna, K., Brindha, P., Lakshmanaperumalsamy, P. 2002. Antimicrobial and phytochemical studies of Swertia corymbosa. Fitoterapia 73:160-164.

[35] Doughari JH. Antimicrobial activity of Tamarindus indica Linn. Trop J. Pharm. Res. 2006; 5: 597.

[36] Liu RH. Potential synergy of phytochemicals in cancer prevention: mechanism of action. J. Nutr. 2004; 134: 3479S-3485S.

[37] Cowan, M.M. 1999. Plant Products as Antimicrobial Agents. Clinical Microbiology Reviews 564-582.

[38] De, M., Krishina De A., and Banerjee, A.B. 1999. Antimicrobial screening of some Indian spices. Phytother Res. 13: 616-618.

[39] Sodipo, O.A., Akiniyi, J.A., and Ogunbanosu. 2000. Studies on certain characteristics of extracts of bark of Pansinystalia macruceras (K.Schem) Piere. Exbeile.Global J. Pure Appl. Sci. 6: 83-87.

[40] Ogunleye DS, and Ibitoye SF. 2003. Studies of antimicrobial activity and chemical constituents of Ximenia americana. Trop. J. Pharm. Res. 2(2), 239-241.