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Antifungal activity of the seed and tuber extracts of *Gloriosa superba* L.

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Abstract: ***Gloriosa superb*** **L. under the family Colchicaceae, commonly called Glory lily, is a medicinally important endangered herbaceous, perennial climbing medicinal plant with L or V-shaped/ finger-like tubers that are pure white when young, becoming brown with age found throughout India. The plant commonly used in several native systems of medicine for the practice of various human diseases like cancer, gout, ulcer, piles, scrofula and act as antipyretic, antihelmintic, purgative, and anti-abortion. But, it also caused disorders and even mortalities to humans and animals due to both purposeful and accidental poisoning. The plant currently widely cultivated throughout the world as an ornamental plant. The better antifungal zone of inhibition observed in 26.3±0.22 mm against *A. flavus* on 5 mg/ml concentration of seed extract followed by 26.0±0.83 mm against *G. fujikuroi* on 10 mg/ml. The minimum zone of inhibition 14.7±0.14 mm in 5 mg/ ml against *C. glabrata* and 16.3±0.53 mm in 10 mg/ml against *F. proliferatum* on 10 mg/ml concentration of methanol extract. Similarly, the maximum zone of inhibition observed in 32.1±0.38mm against *A. foetidus* on 10 mg/ml concentration of tuber extract followed by 31.0±0.21 mm against *A. ustus* on 10 mg/ml. The minimum *zone of inhibition* 17.0±0.01mm *observed* against *F. proliferatum* on 5 mg/ml concentration of methanol extract followed by 23.0±0.01mm against *Candida albicans.* The antifungal activity of the tuber has a good source of antifungal fungal substance than seed methanol extract. The study confirmed that the methanol tuber extract is having a good source of antifungal content than seed methanol extract and to be isolated the secondary metabolites and also to be developed new drugs against *A. foetidus, A. niger, T. viride* and *A. ustus* from the tuber.**

Keywords: ***Gloriosa superb,*** **seed, tuber, antifungal activity, methanol extract.**

I. INTRODUCTION

Human beings have been utilizing plants for primary health care since time immemorial. Recent estimates reported that over 9,000 plant species had known medicinal values in various cultures and countries and without having conducted comprehensive research amongst several indigenous and also other communities. In India, approximately 1700 plant species used in Ayurveda, 500 for Siddha, 400 for Unani, 300 for Amchi systems of medicine with substantial overlaps of common plants among these systems. The Colchicaceae family has several members that are of economic importance. Notable among them are *Colchicum autumnale* L., *C. luteum* Baker, and *Gloriosa superb* grew as sources of colchicine [1]. [2] reported *G. superba* exported by India, Sri Lanka, Nigeria, Cameroon, and Zimbabwe to pharmaceutical industries. Colchicine is an ancient anti-inflammatory medicine, has been employed newly for treating an increasing number of disorders characterized by enhanced leukocyte trafficking including Behçet's syndrome, primary biliary cirrhosis, alcohol-induced liver cirrhosis, psoriasis, Sweet's syndrome, scleroderma and sarcoidosis [3];[4]. Since 1972, colchicine has become the drug for prophylaxis against Familial Mediterranean Fever (FMF) attacks and risk of amyloidosis[3];[4].

1.1 Traditional uses of Gloriosa superba

Gloriosa superba L. under the family Colchicaceae, commonly called Glory lily, is a medicinally important endangered herbaceous, perennial climbing medicinal plant with L or V-shaped/ finger-like tubers that are pure white when young, becoming brown with age found throughout India. The plant currently widely cultivated throughout the world as an ornamental plant [5]The plant commonly used in several native systems of medicine for the practice of various human diseases like cancer, gout, ulcer, piles, scrofula [6]and act as antipyretic, antihelmintic, purgative and anti-abortion. But, it also caused disorders and even mortalities to humans and animals due to both purposeful and accidental poisoning. Traditionally the tubers and leaves of the plant are commonly being used in the treatment of abdominal pain, anthelmintic, bruises, infertility, inflammation, laxative, leprosy, parasitic skin infections, piles, ulcers,[7]. In Folklore, it is being used to treat baldness, killing lice in the hair, pimples, skin eruptions, and also as a sedative [8].

The root tuber ground with seeds of *Psoralea corylifoila*, *Nigella sativa* (black cumin) and *Vernonia anthelmintic* (purple fleabane) and made to a paste. The past is applied externally for various skin diseases [9]. Usually, the rural women prefer *G. superba* roots and rhizomes are used in traditional system of medicine for using gynecological disorders like abortion, conception disorders, delivery problems, menstrual trouble, sterility, etc. rather than modern drugs. It is very important ethnomedicine to the tribal's. In Madhya Pradesh, the Gond tribe used orally for inducing abortion; they grind rhizome/tuber of *G. superba* mixed with ghee [10]. The tribes of Deogarh district used tuber against piles and using herbal medicines for their primary health care [11]. The rhizome is anthelmintic, anticancerous, antileprotic, antimalarial, cholagogue, febrifuge, oxytocic, purgative, and stomachic. The leaf is anti-inflammatory and antiasthmatic. Root shows antibiotic activity and antigonorrhoeic. Even the leaves have more medicinal qualities for curing asthma, the juice is effective against lice and also many skins and various respiratory disorders[12].

G. superba used for curing an extensive range of human ailments all over the World. In India, the Ayurvedic Pharmacopoeia recommends *G. superba* as an ecbolic in labor, an anthelminthic, chronic ulcers, colics, cure against leprosy, haemorrhoids, head lice, purgative, skin-parasites and tumours [5]; [13]; [14];[15];[16]; [17]; [18]; [19]; [10]. The tuberous of *G. superba* is boiled with *Sesamum* oil and practice twice a day on the joints as a therapy against arthritis, and to reduce pain [20]. The leaf extract is used as a smoothening agent for skin complaint [21]. (Hemaiswarya *et al.*, 2009). Seeds are used for muscle relaxant and relieving rheumatic pain [22]. Traditionally, the watery extract of tuber has been used as an abortifacient [23]; [2];[24]; [25]; [15];[26]; [17]; [18], as a cure against venereal diseases[2]; [17];[27] abdominal and general body pain [2];[25] 1964; [26] [17]. The tubers tied around doors and windows to prevent the entry of snakes; and also used as an antidote of scorpion sting and snake bite [9].

Five different plant parts of *G. superba* leaves, seeds, unripe fruit, the rootstock or tuber, and the whole plant quoted as necessary in ethnobotanical applications. The tuber or rootstock is the plant part that most frequently used as medicine[2]; [17]. Five different pharmaceutical methods were cited: comprising paste, decoction (extraction in hot water), maceration (soaked in cold water), powder, and using the whole plant without specific formulation. The decoction and maceration used for the internal body ailments, like abdominal pain[2];[25]; [26]; [17];[28],coughs [2];[25]; [17], fever and malaria [24]; [29] etc. Tuber paste of *G. superba* is applied externally to cure venereal diseases [2]; [17]; [27], parasitic skin diseases [2];(Dounias 2006;[30] Hassan and Roy, 2005; [31] Watt and head lice[23];[25];[17];[31], and wounds [23]; [2];;[25]; [30]; [16]; [17].

Nowadays, *Gloriosa superba* widely cultivated for commercial purposes. It contains the alkaloid colchicine, which used in the manufacture of drugs. Colchicine has been used efficiently in the treatment of numerous diseases such as abortifacient, acute gout, an antidote to a snake bite, bruises, infertility, intestinal worms, laxative, nails, removal of thorns, skin parasites, skin problems, and spines. It has also confirmed for the treatment of arthritis, cholera, chronic ulcers, colic, kidney problems, and typhus [9]. Colchicine is generally used as an investigational tool for the study of cell division in research, as it can stop mitosis, induce polyploidy, and also used in the treatment of cancer.

1.2 Uses of Colchicine

Nowadays, *Gloriosa superb* widely cultivated for commercial purposes. It contains the alkaloid colchicine, which used in the manufacture of drugs. Colchicine has been used efficiently in the treatment of numerous diseases such as abortifacient, acute gout, an antidote to a snake bite, bruises, infertility, intestinal worms, laxative, nails, removal of thorns, skin parasites, skin problems, and spines. It has also confirmed for the treatment of arthritis, cholera, chronic

ulcers, colic, kidney problems, and typhus [9]. Colchicine is generally used as an investigational tool for the study of cell division in research, as it can stop mitosis, induce polyploidy, and also used in the treatment of cancer.

1.3 Awareness of *G. superba*

The toxins of the plant have an inhibitory action on cellular division resulting in diarrhea, the depressant effect on the alopecia and bone marrow. Usually, all parts of the *G. superba*, especially the tubers are incredibly poisonous [32];[33] and burning sensation, cause vomiting, purging, stomach ache [34] **(**Roberts *et al*., 1987**).** The glory lily has used for suicidal purposes in India, Burma, and Eastern Africa due to the presence of colchicine [35];[36].

II. MATERIALS AND METHODS

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

2.1 Successive extractive value

The seeds and tubers were allowed to dry in shade and coarse powder. The coarse powders were extraction with methanol using a Soxhlet apparatus[37].

2.2 Determination of Antimicrobial Activity

The antifungal activity of seed and tuber methanol extracts of *G. superba* determined by agar well diffusion method [38]. The various concentrations of 10 and 5mg/ml methanol extracts of seed and tuber prepared for antimicrobial activity.

2.3 Test fungus

Thirteen human pathogenic fungal strains *Aspergillus flavus, Aspergillus foetidus, Aspergillus niger, Aspergillus ustus, Candida albicans, Candida glabrata, Fusarium oxysporum, Fusarium proliferatum, Fusarium tricinctum, Giberella fujikuroi, and Trichoderma viride* procured from National Collection of Industrial Microorganisms, National Chemical Laboratory, Pune. The organism used for antifungal studies. The organism was kept at 4°C on agar slant and sub-cultured at 37°C for 24 hrs in Potato dextrose agar for fungus before *in vitro* susceptibility tests. Agar well diffusion method [38] adapted to determine the antimicrobial activity. Potato dextrose agar plate seeded with (Sterile cotton swabs) a spore suspension of 16 hours old broth culture of particular fungus. 8 mm diameter of two wells were made in each of these plates using sterile cork borer, and about 0.3 ml of 5 and 10 mg/ ml concentration of seed and tuber methanol extracts were added using sterilized dropping pipettes into the wells and allowed to diffuse at room temperature for 2 hours. The plates were incubated three days at 37ºC for fungal pathogens, and proper control of solvent extracts was also maintained. The diameter of the inhibition zones recorded. Triplicates kept, and the experiment repeated thrice, and the average values recoded for antimicrobial activity.

2.4. Statistical Analysis

Statistical analysis was done using the GraphPad Instat Statistics Version 2. The processing of raw data collected from the experiments and getting mean value and SD of the sample.

III. RESULTS AND DISCUSSION

3.1 Antifungal activity of methanol extract of the tuber

Maximum zone of inhibition observed is 32.1±0.38mm against *Aspergillus foetidus* when applied 10 mg/ml concentration of tuber extract, followed by 31.0±0.21 mm against *Aspergillus ustus* at 10 mg/ml (Table 1; Graph.1). Minimum zone of inhibition of 17.0±0.01mm was recorded against *Fusarium proliferatum* when used at 5 mg/ml concentration of methanol extract followed by 23.0±0.01mm against *Candida albicans.* Better antifungal zone observed at a high level (10 mg/ml) of methanol extract than lower concentrations. This study denoted that the high concentration of extract has more antagonistic of activity and active principle than more moderate levels of the extract.

3.2 Antifungal activity of methanol extract of seed

Best antifungal activity and zone of inhibition were observed as 26.3±0.22 mm against *Aspergillus flavus* at 5 mg/ml concentration of seed extract followed by 26.0±0.83 mm against *Gibberella fujikuroi* at 10 mg/ml (Table.1; Graph. 1).

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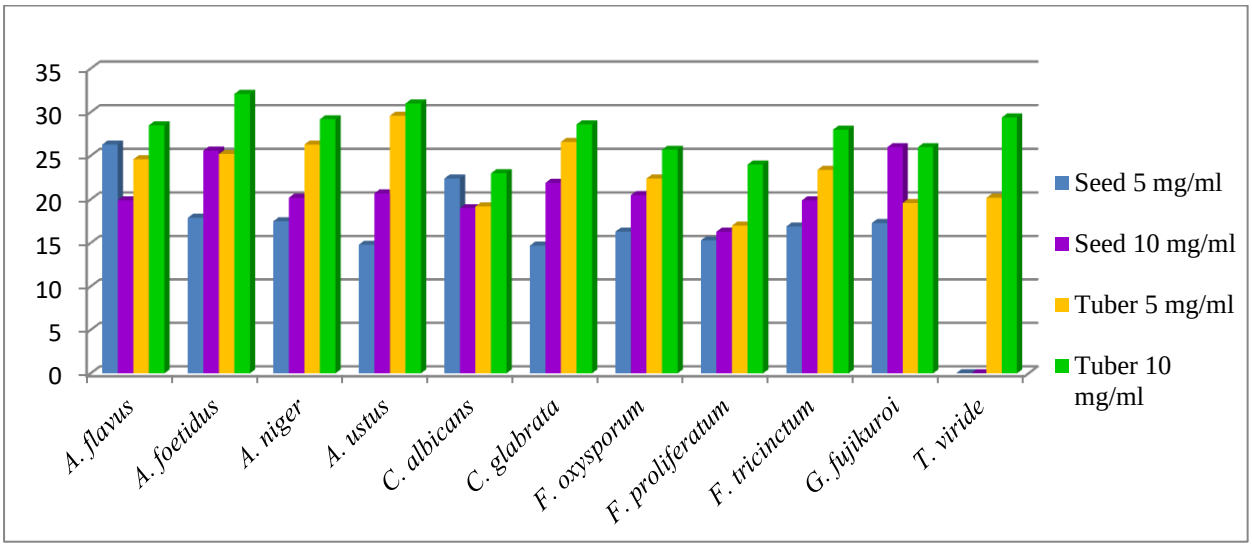
Lowest zone of inhibition, *i.e.*, 14.7±0.14 mm recorded when used in 5 mg/ml concentration against *candida glabrata* and 16.3±0.53 mm at 10 mg/ml against *Fusarium proliferatum* at 10 mg/ml concentration of methanol extract. *G.superba* tuber has a good source of antifungal substance than seed methanol extract.

The tuber and seed extract shows vigorous antifungal activity against tested organisms. Comparative survey against of bacterial and fungal pathogens revealed that crude extracts of *G. superba* tubers in methanol showed better activity against fungal pathogens than bacterial counterparts (Table.1; Graph.1). Among the organisms, *A. foetidus and A. ustus* were inhibited maximum by tuber extracts. This research is in a strong opinion that methanol extracts have potent antifungal activity and those are to be isolated and identified active compounds, which in turn can be characterized and developed in new and safe drugs against fungal diseases. Methanol extracts having the capacity to control plant pathogen of all *Fusarium species.* The seed extract did not show any activity against *T. viride*, but the tuber extract predominantly controls to *T. viride.* The *T. viride* is well known as a biopesticide, which acts against the bacterial and fungal pathogens in the field conditions. But this study proved that the tuber extras work against *T. viride*. So, the application of T. *viride* in a field where *G. superba* is grown earlier to control bacterial and fungal pathogens may not have much effect due to the metabolizes or resides of *G. superba* present in the soil.

Table-1 Antifungal activity of methanol extract of *G. superba* tuber and seed

Organism	NCIM Acc. No	Seed extract mg/ml		Tuber extract mg/ml	
		5	10	5	10
A .flavus	1028	26.3±0.22	19.9±1.33	24.6±0.07	28.5±0.24
A.foetidus	1027	17.9±0.04	25.6±0.27	25.2±0.34	32.1±0.38
A. niger	1004	17.5±0.31	20.2±0.37	26.3±0.36	29.2±0.13
A. ustus	1033	14.8±1.42	20.7±0.36	29.6±0.76	31.0±0.21
C.albicans	3100	22.4±0.71	19±0.25	19.2±0.66	23.0±0.01
C. glabrata	3237	14.7±0.14	21.9±1.04	26.6±0.07	28.6±0.07
F. oxysporum	1008	16.3±0..52	20.5±0.88	22.4±0.14	25.7±0.92
F. proliferatum	1103	15.3±0.86	16.3±0.53	17.0±0.01	24.9±0.39
F. tricinctum	1189	16.9±0.75	19.9±0.54	23.4±0.82	28.0±0.82
G. fujikuroi	1036	17.3 ±1.04	26.0±0.83	19.6±0.27	26.0±0.62
T. viride	1051	-	-	20.2±0.46	29.4±0.62

* Zone of inhibition in mm



Graph.1: Antimicrobial and of *G. superba* tuber extracts vs. seed extract

Medicinal plants are the primary sources of new drug and may initiate an alternative to the usual medicines. The therapeutic and aromatic plants are used on a vast, full scale in medicine against drug-resistant bacteria [39]. The *G. superba* is a popular ethnomedicine in Indian system of medicine for its colchicine content which is used to treat arthritis, acute gout, an antidote to a snake bite, infertility, intestinal worms, laxative, skin parasites, skin problems, spines and treatment of cancer. Phytochemical studies of tubers having colchicines, glycoside, gloriosine, flavonoids, tannins, alkaloids, 3-Odemethylcolchicine- 3-O-α-D-glucopyranoside, 1,2- didemethyl colchicine, β and γ Lumicolichicines, β sitosterol, glucoside, 2,3-didemethyl colchicine, luteolin, N-formyldeacetyl colchicines, colchicoside, tannins, superbine, 2-hydroxy-6-methoxy benzoic and salicylic acid [40];[41]. In our study the phytochemicals of tuber having less activity against *C.albicans*, but this study is disagreement to earlier report said that the better inhibitory zone observed against selected human pathogen including *E. coli* because of the influence of phytochemical from the tuber [42];[43];[44]. The antimicrobial activity of the extracts depends on a lot of factors like the binding capacity, chelation of iron, and proteins of the bacterial cell membranes and antibacterial mechanisms of the phytochemicals [45]. This study is controversy to an earlier report, state that the antimicrobial activities of 75% methanol extract from *A. paniculata* leaves were observed only against the *S. aureus* [46];[47]. In our study, the low concentration of methanol showed a better zone of inhibition against the tested pathogen. This result is similar to the earlier observation of many researchers [48];[49];[50];[47];[51];[51]. All the extracts of *G. superba* to control both gram-positive and negative organism [44] this study passively correlated with earlier observation in *Andrographis paniculata, Bridelia crenulata, Begonia malabarica, Swertia corymbosa, Drynaria quercifolia* [52]; [53];[54];[55].

The naturally arising alkaloids have nitrogenous compounds that institute the essential phytochemicals of flowering plants. Alkaloids are formed as metabolic products and have described being accountable for pharmacological value [56]. Alkaloids have identified in the extracts or compounds that have been documented to possess medicinal properties and to promote health effects [57];[58]. Glycosides are served as defense mechanisms against predation by microbes, insects, and herbivores [59]. These compounds served as essential drugs, which help the body to fight microbial infections [60]. Tannins have been used traditionally for protection of wound on surfaces of the mouth and treatment of catarrh, diarrhea, and hemorrhoids. Plant tannins also accepted for their pharmacological properties[61]. In the assessment of the previous and present results, it is clear that the plant maintains the antimicrobial property of the methanol extracts of the tuber and *G. superba*. The microbial effect of the raw tuber extracts is better than the standard antibiotic drugs Ampicillin and Tetracycline [51]. The present study denoted that tuber has an enormous active principle to give the more antifungal effect and to be isolated the secondary metabolites and also to be developed new drugs against *A. foetidus* and *A. ustus* of fungal infections.

IV. CONCLUSION

The antifungal activity of the tuber has a good source of antifungal fungal substance than seed methanol extract. The study confirmed that the methanol tuber extract is having a good source of antifungal content than seed methanol extract and to be isolated the secondary metabolites and also to be developed new drugs against *A. foetidus, A. niger, T. viride* and *A. ustus* from the tuber.

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