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Eficacy of Gloriosa Superba L. On Plant Pathogenic Fungi

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Abstract

Gloriosa superba is a endangered toxic plant belong the family Colchicaceae (Liliaceae) commonly known as kalihari (Hindi), glory lily (English) containing high levels of colchicines and gloriocine both are toxic alkaloid. Traditionally it is used for the treatment of various diseases. The aim of present research screening of phytochemical profile and evaluate the Antifungal activity of Gloriosa superba against four plant pathogenic fungal spp. namely Fusarium oxysporum, Alternaria species, Sclerotium rolfsii and Rhizoctonia solani. These selected fungi causes a wide range of commercially significant plant disease; some time also in animals and human. To determine the antifungal activity, food poisoning method was employed. Different concentration of methanoic extract (6.25, 12.5, 25, 37.5, 50, 62.5 µg/ml) of Gloriosa superba were tested against fungal strain and the phytochemical screening were perform by standard harbone method. The methanol extract of Gloriosa superba L. showed very good antifungal activity against selected plant pathogen fungi. The excellent inhibitory activity was observed against Rhizoctonia solanii (99.33%) and Alternaria sp. (98.13%) followed by Sclerotium rolfsii (84.26%) and Fusarium oxysporum (63.23%) at the concentration of 62.5 µg/ml. The results of the phytochemical screening revealed the presence of alkaloids, steroid, carbohydrates, tannins, phenolic compounds, terpenoid, cardiac glycoside, flavonoids and absence of phlobotannin, saponin, anthraquinone. It may be due to presence of phytochemicals like colchicines (alkaloid) plant show antifungal activity. The excellent inhibitory results conclude that the crude plant extact can be used as alternative of biopesticide and some antifungal drugs.

Keywords: *Gloriosa superba*, antifungal activity, phytochemical screening, food poisoning method.

Introduction

Plants form the main ingredients of medicines in traditional systems of healing and have been the source of inspiration for several major pharmaceutical drugs. Since prehistoric times, plants and their extracts have been used for their healing properties.

Among Ancient civilizations, India has been known to be rich in Medicinal Plant. *Gloriosa superba* is a one of the Medicinal important species of flowering plant of Colchicaceae family. It is also known as Glory lily, gloriosa lily (English language); Kalihari (Hindi). This species is a perennial herb growing from a fleshy rhizome. It is scandent, climbing using tendrils, the stem reaching 4 meters long. The leaves are mainly alternately arranged, but they may be opposite, as well[1].

It is a toxic in nature; containing high amount of colchicine (a toxic alkaloid) and goriocine. As the toxic syndrome progresses, rhabdomyolysis, ileus, respiratory depression, hypotension, coagulopathy, haematuria, altered mental status, seizures, coma, and ascending polyneuropathy may occur[2].

Fusarium is a large genus of filaments fungi widely distributed in soil. The name *Fusarium* comes from lation fusus, meaning a spindle. The colour of colonies may be white, salmon, cinnamon, red, violet, pink or purple. *Fusarium* generally produces symptoms such as wilting, chlorosis, necrosis, premature leaf drop, browning of the vascular system, stunting and damping off [3].

Alternaria is a genus of ascomycete fungi. *Alternaria* species are known as major plant pathogens. *Alternaria* species are a leading cause of crop blight and they cause allergies and infection in some people and animals. Garden vegetables likely have *Alternaria* infection if round or angular spots show up on plant leaves. These spots may take on a target-like appearance with a dark edge around the spots or they may be completely black. The affected leaves soon winter and drop off eventually the entire plant dies[4].

Scleratium is a compact mass of hardened fungal mycelium containing food resources. sclerotia contain alkoloies that when consumed can cause ergotism with is a disease that cause loss of peripheral sensation twitches and loss of affected tissues. S. *rolfsii* attacks stems, roots, leaves, and fruit. In sweet potato beds, white mycelium is reported to cover the soil surface and grow up and over sprouts. sclerotia may exist free in the soil or in association with plant debris[5].

Rhizoctonia was introduce in 1815 by French mycologist Augustin Pyramus de candolle for plant pathogenic fungi that produce both hyphae and sclerotia. *Rhizoctonia* means “root

killer". *Rhizoctonia solani* is a plant pathogenic fungus with a wide host range and worldwide distribution. It is best known to cause various plant disease such as collar root, damping off, wire stem and root rot. *Rhizoctonia solani* causes a wide range of commercially significant plant disease. It is one of the fungi responsible for brown patch. bare patch of cereals, root of sugar beet, sheath blight of rice and many other pathogenic condition. *Rhizoctonia solani* attacks its hosts at seedlings stages, which are typically found in the soil, their main targets are herbaceous plants[6].

Material and Method

Collection of plant materials: Fresh tuber of *Gloriosa superba* were collected from Chainpur, Gumla, Jharkhand and identified by Botany department, Ranchi University, Ranchi. The plants were washed thoroughly 2-3 times with running water and once with sterile distilled water, dried in shade.

Solvent extraction: The methanolic extracts was prepared from the dried powder of plant tuber mix with desirable amount of methanol and placed in shaker incubator for 48 hours. The extract was filtered; the filtrate obtained was dried and stored at 4°C for further process.

Microbial species: The antimicrobial activities of plant extract were investigated against four plant pathogenic fungal species *Fusarium oxysporium*, *Alternaria sp.*, *Rhizocotonia solani* and *Sclerotium rolfsii*. Pure culture of all the fungal and bacterial species are already maintained in Laboratory of Plant Physiology and Biotechnology, University Department of Botany, Ranchi University, Ranchi.

Preparation of the PDA medium: The potato slice were boiled with a little amount of distilled water for 30 minutes and applied for coarse filtration by the help of filter paper. The required amount of dextrose and agar were properly mixed with filtrate. After adjustment of pH, finally make up the volume by adding distilled water. After autoclave, mix 250 mg/L of streptomycin to inhibit the growth of bacteria.

Antifungal assay: in vitro antifungal activity of plant extract were tested against four fungal species by food poisoning method where Potato Dextrose Agar (PDA) medium was used for the growth of fungus. Different concentrations 6.25µg/ml, 12.5 µg/ml, 25 µg/ml, 37.5 µg/ml, 50 µg/ml, 62.5 µg/ml of plant extracts were mixed with fungal medium after autoclaving. The activity was determined to measure the growth of fungal mycelia on control (no extract) and test sample with the help of scale at regular interval.

Phytochemical Screenings: The plant extract was analyzed for the presence of flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides and tannins according to standard methods[7].

Test for tannins: About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for browrish green or a blue-black colouration.

Test for phlobatannins: Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatinins.

Test for saponin: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for flavonoids: 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4. A yellow colouration observed in each extract indicated the presence of flavonoids.

Test for steriods: Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H_2S0_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for terpenoids (Salkowski test): Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for cardiac glycosides (Keller-Killani test): Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Test for phenols: Crude extract was mixed with 2ml of 2%solution of $FeCl_3$. A blue green or black coloration indicated the presence of phenols.

Test for tannins: Crude extract was mixed with 2ml of 2% solution of $FeCl_3$. A blue – green or black colouration indicated the presence of tannins.

Test for anthraquinones: Borntrager's test was used for detecting the presence of anthraquinones. In this case 0.5 g of the plant extract was shaken with benzene layer separated and half of its own volume of 10% ammonia solution added. A pink, red or violet colouration in the ammoniacal phase indicated the presence of anthraquinone.

Results and Discussion

Antifungal assay: To determine the antifungal activity, food poisoning method was used. Different concentration of methanolic extract (6.25, 12.5, 25, 37.5, 50, 62.5 µg/ml) of *Gloriosa superba* were tested against four plant pathogenic fungal strain. The methanol extract of *Gloriosa superba* L. showed very good fungal inhibitory activity. The excellent inhibitory activity was observed against *Rhizoctonia solanii* (99.33%) and *Alternaria* sp. (98.13%) followed by *Sclerotium rolfsii* (84.26%) and *Fusarium oxysporum* (63.23%) at the concentration of 62.5 µg/ml. Among different fungi tested *Rhizoctonia solanii* and *Alternaria* sp. were found to be more sensitive when compare to *Sclerotium rolfsii* and *Fusarium oxysporum*.

Phytochemical screening: The results of the phytochemical screening revealed the presence of alkaloids, steroid, carbohydrates, tannins, phenolic compounds, terpenoid, cardiac glycoside and flavonoids and absence of saponin and phlobotanin.

Table-1
Phytochemical screening of *Gloriosa superba*

Sl.No.	Phytochemical	Observation	Result
1.	Tannin	Brownish black ppt	Present
2.	Phlobotannin	Red precipitate formed	Absent
3.	Terpenoid	A reddish brown colour formed	Present
4.	Saponin	Froathing not observed	Absent
5.	Flavonoid	Yellow colour	Present
6.	Cardiac glycoside	Ring formed	Present
7.	Phenol	Reddish black	Present
8.	Steroid	Red colour observed	Present
9.	Alkaloid	Turbidity obtained	Present
10.	Anthraquinone	Pink colour not observed	Absent
11.	Carbohydrate	Black colour not observed	Present

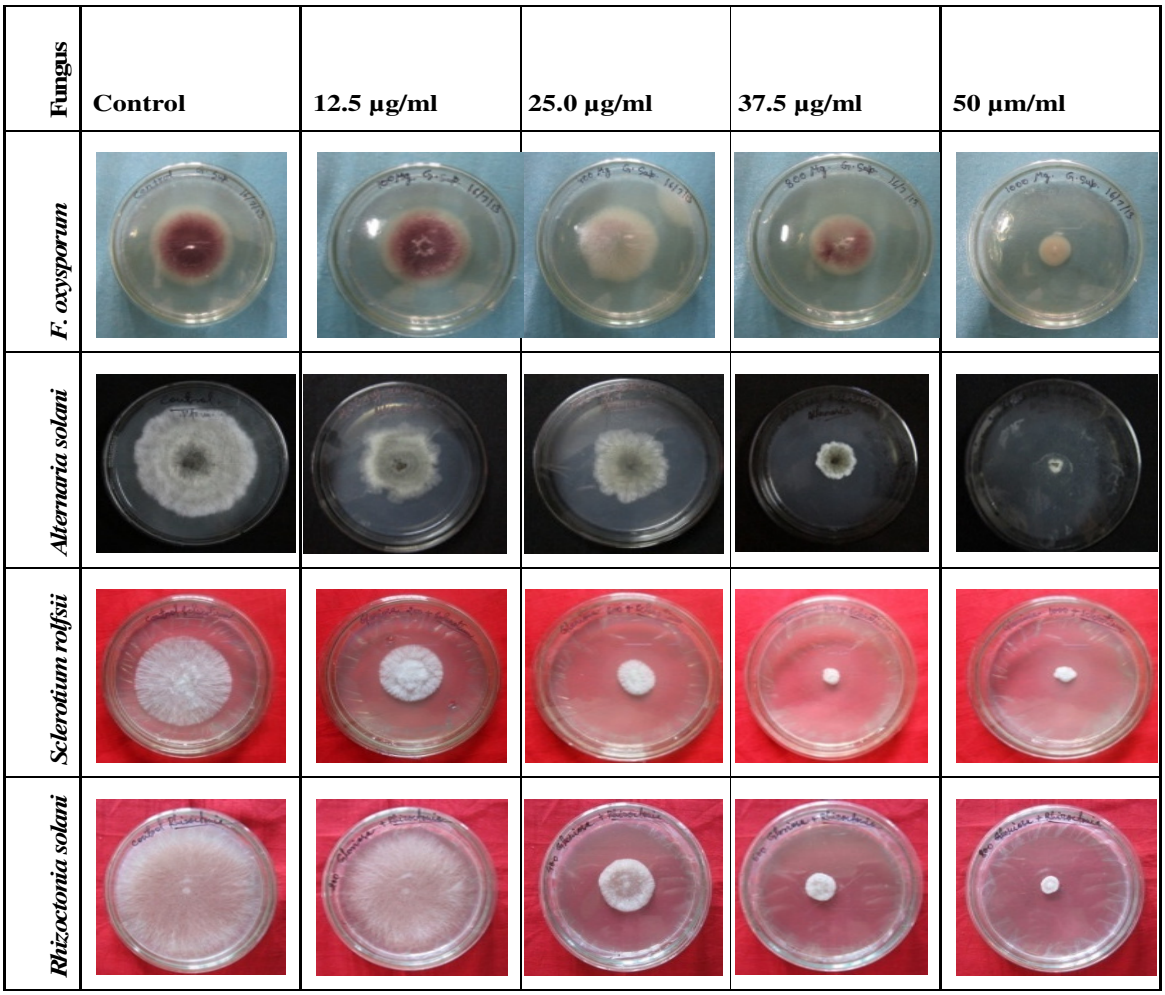

Figure-1
Show effect of different concentration of plant extracts on four pathogenic fungus

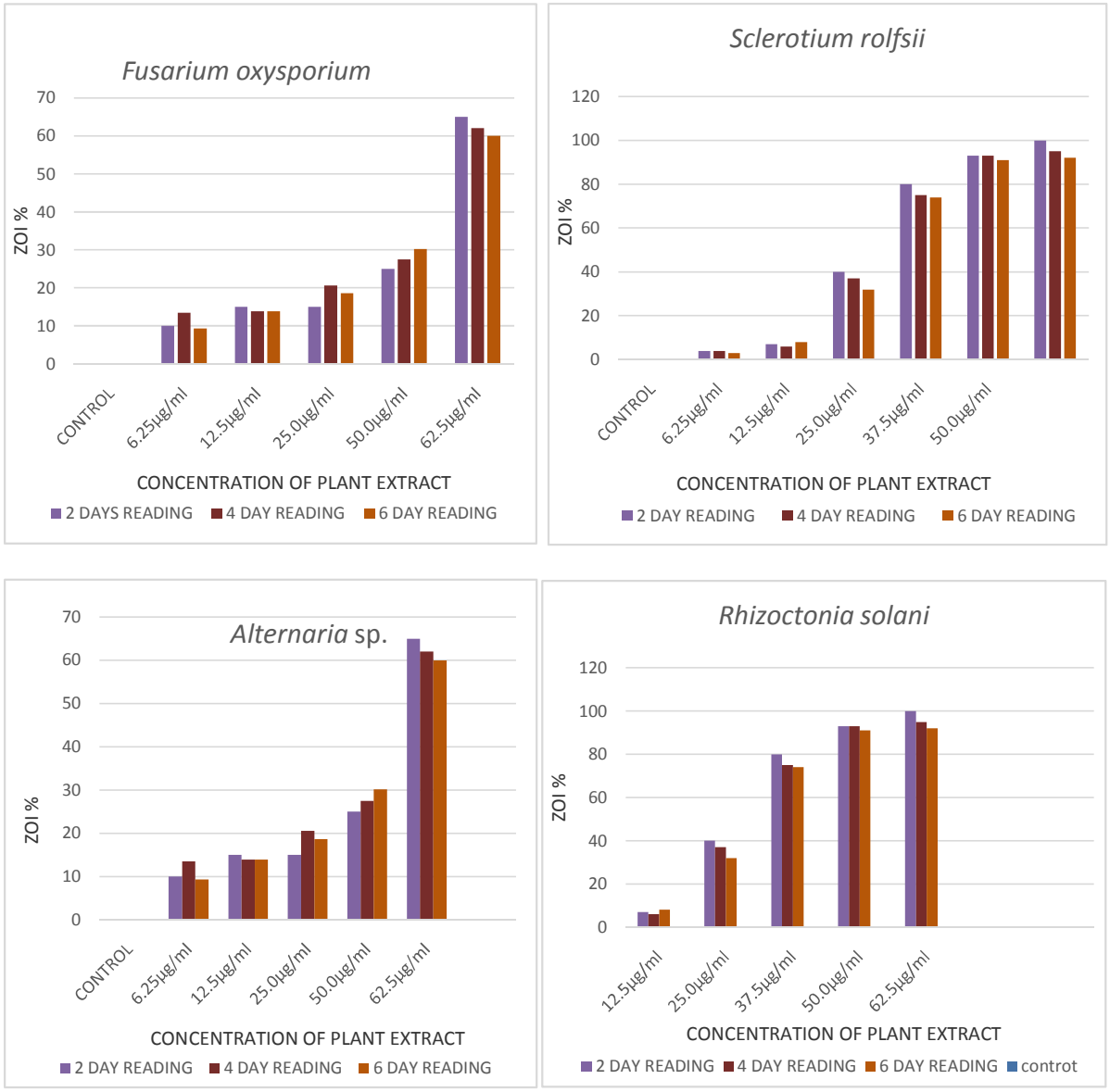

Figure-2
Showing effect of *Gloriosa superba* extract against some Fungus

Discussion: Folk medicine is first hand source of information about the therapeutic efficacy of phytochemicals against different kinds of diseases. The literature indicates that the antibacterial activity is due to different chemical agents present in the extract, including essential oils (especially thymol), flavonoids and triterpenoids and other natural phenolic compounds or free hydroxyl groups. These are classified as active antimicrobial compounds[8].

However, the methnolic extract (Carbohydrates, Glycoproteins, cinnamic derivatices and leococyanidines) has showed the presence of biologically active compounds correlated to known substance that possess antimicrobial properties[9,10]. The study provides strong circumstantial evidence that small protein or peptide present in the plant extract will play an important role in plant's antimicrobial defense system[11]. Similar antimicrobial studies have been done by several workers[12-15]. Antimicrobial activities have been attributed to different phytochemicals.

Conclusion

Nature has produces a lot of medicinal agents for thousands of years and an impressive number of modern drugs contain isolated from natural resources. Recent, attention has been paid to biologically active extracts and compounds from plant species used in herbal medicines. In addition, the knowledge of the chemical constituents of plants would ferther be valuable in discovering the actual value of falkloric remedies.

Table-2
Efficacy of different concentration of plant extracts on four pathogenic fungus

Fungus name	Concentration	Zone of fungal mycelia spreading (In cm)				
		3ed DAY	4th DAY	5th DAY	6th DAY	Average ±SD
Fusarium oxysporum	CONTROL	2	2.9	3.6	4.4	3.225±1.02
	6.25 µg/ml	2	2.8	3.4	4.1	3.075±0.89
	12.5µg/ml	1.9	2.7	3.2	3.9	2.925±0.84
	25 µg/ml	1.7	2.5	3.1	3.7	2.75±0.85
	37.5 µg/ml	1.6	2.3	2.8	3.4	2.525±0.76
	50 µg/ml	1.5	2.0	2.6	3.0	2.275±0.66
	62.5 µg/ml	0.7	1.1	1.3	1.6	1.175±0.37
Sclerotium rolfsii	CONTROL	2.0	4.7	6.2	6.4	4.825±2.03
	12.5µg/ml	1.7	3.2	5.1	5.7	3.925±1.82
	25 µg/ml	1.1	2.8	4.2	4.9	3.25±1.67
	37.5 µg/ml	0.7	2.1	3.7	4.2	2.675±1.59
	50 µg/ml	0.3	1.2	1.9	2.2	1.4±0.84
	62.5 µg/ml	0	0	0.2	0.5	0.175±0.23
Rhizoctonia solani	CONTROL	9	9.6	11.0	11.8	10.35±1.27
	12.5µg/ml	5.5	7	7.3	9.1	7.225±1.47
	25 µg/ml	2.7	3.5	4.2	5.5	3.975±1.18
	37.5 µg/ml	1.1	1.4	1.6	2.3	1.6±0.50
	50 µg/ml	0.3	0.4	0.5	0.7	0.475±0.17
	62.5 µg/ml	0	0	0	0	0±0
Alternaria sp.	CONTROL	1.2	4.5	6.4	6.7	4.7±2.52
	6.25µg/ml	0.9	3.6	5.2	6.4	4.025±2.37
	12.5µg/ml	0.9	2.8	4.4	5.1	3.3±1.86
	25 µg/ml	0.6	2.2	3.3	4.0	2.525±1.48
	37.5 µg/ml	0.40	1.3	2	2.3	1.5±0.84
	50 µg/ml	0	0.4	0.6	0.7	0.425±0.30
	62.5 µg/ml	0	0	0.1	0.2	0.075±0.09

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