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PHYTOCHEMICAL, *IN VITRO* ANTIBACTERIAL AND ANTIFUNGAL ANALYSIS OF LEAF EXTRACTS OF *SOLANUM ERIANTHUM* D. DON

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ABSTRACT

The role of natural products as a source for remedies has been recognized since the beginning of mankind. Nevertheless, a minority of folkloric used medicinal plants have been evaluated for their pharmacological activities. The purpose of this study is to evaluate the in vitro antimicrobial, antifungal and phytochemical analysis of the medicinal plant of *Solanum erianthum* D. Don. Antibacterial activity in *S. erianthum* was performed for six organisms. The highest performance is seen in *Bacillus cereus* with 40µg concentrations and is higher than the control. Antifungal activity in *S. erianthum* was performed with six organisms. The highest activity is seen in *Botrytis cinera* and the activity is higher the control. The phytochemical analysis was carried out for the solvents Acetone, Ethyl acetate and Hydro alcohol solvent extracts. More constituents are found in hydro alcohol extracts. The constituents alkaloids, flavonoids, phenols, saponin, tannin and carbohydrates are seen in hydro alcohol extract.

Keywords: *Solanum erianthum D.Don,* Phytochemical, Anti-bacterial, and Anti-fungal.

INTRODUCTION

Plants have been used as a folkloric source of medicinal agents since the beginning of mankind. Despite major scientific progress in chemistry, drugs derived from plants still make an enormous contribution to drug discovery today and continue to be an important source to fight serious diseases, especially in developing countries (Zhang, 2004). Infections caused by microorganisms and parasites as well as malignant diseases are still a serious threat to public health, despite the great development in modern medicine. The relative unavailability of medicines in developing countries and the current problems associated with the use of

antibiotics makes the impact of infectious diseases particularly large and significant (Okeke *et al*., 2005). Thus, the interest in herbal drugs with antimicrobial properties has been revived. With the increasing prevalence of multi-resistant bacteria, the search for plant extracts against these organisms offers an important potential for the development of new agents effective against infections which are difficult to treat (Cowan, 1999; Rios & Recio, 2005).

More than 800 million people in the developing countries do not have adequate food supplies and at least 10% of food is lost due to plant diseases (Strange and Scott, 2005). As compared to other plant parasites, fungi cause the greatest impact with regard to diseases and crop production losses. The most important method of protecting the plants against the fungal attack is the use of fungicides. However, many fungicidal agents available in the market are toxic and have undesirable effects on other organisms present in the environment. Some synthetic fungicides are non-biodegradable, and hence can accumulate in the soil, plants and water, and consequently affect the humans through the food chain. The development of resistance of pathogenic fungi towards the synthetic fungicides is of great concern. Therefore, it is desirable to use some eco-friendly measures for the management of diseases.

MATERIALS AND METHODS

Collection and identification of the plant material

The fresh plant leaves of *S. erianthum* were collected randomly from Yercaurd, Salem District, Tamilnadu, in the month of November 2010. The plant was authenticated by Dr. S.John Brito S.J.Rapinat herbarium and Centre for Molecular Systematics, St. Joseph's College (Autonomous), Tiruchirappalli, Tamilnadu, India. A voucher specimen has been retained in our laboratory for future reference (JM.001/2010).Plant materials were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles in refrigerator.

Preparation of Extract

Crude plant extract was prepared by Soxhlet extraction method. About 20gm of powdered plant material was uniformly packed into a thimble and extracted with 250ml of Acetone, ethyl acetate and Hydroalcohol extract separately. The process of extraction has to be continued for 24 hours or till the solvent in siphon tube of extractor become colorless. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40ºC till all the solvent got evaporated. Dried extract was kept in refrigerator at 4ºC till future use.

Phytochemical screening

Preliminary phytochemical analysis was carried out for all the extracts of *S. erianthum* as per standard methods described by Brain and Turner 1975; Evans 1996.

Detection of alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrate was used to test the presence of alkaloids.

a) **Mayer's test**: Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

b) **Wagner's test**: Filtrates were treated with Wagner's reagent. Formation of brown/ reddish brown precipitate indicates the presence of alkaloids.

Detection of Flavonoids

a) **Lead acetate test**: Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

b) **H_2SO_4 test**: Extracts were treated with few drops of H_2SO_4. Formation of orange color indicates the presence of flavonoids.

Detection of Steroids

Liebermann-Burchard test: 2ml of acetic anhydride was added to 0.5g of the extracts, each with 2ml of H_2SO_4. The colour changed from violet to blue or green in samples indicate the presence of steroids.

Detection of Terpenoids

Salkowski's test: 0.2g of the extract of the whole plant sample was mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) and carefully added to form a layer. A reddish brown coloration of the inner face indicates the presence of terpenoids.

Detection of Anthroquinones

Borntrager's test: About 0.2g of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. A Few drops of 10% NH_3 were added to the mixture and heated. Formation of pink colour indicates the presence anthraquinones.

Detection of Phenols

a) **Ferric chloride test**: Extracts were treated with few drops of 5% ferric chloride solution. Formation of bluish black color indicates the presence of phenol.

b) **Lead acetate test**: Extract was treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of phenol.

Detection of Saponins

Froth test: About 0.2g of the extract was shaken with 5ml of distilled water. Formation of frothing (appearance of creamy stable persistent of small bubbles) shows the presence of saponins.

Detection of Tannins

Ferric chloride test: A small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and 0.1% ferric chloride was added to the filtrate. A dark green colour formation indicates the presence of tannins.

Detection of Carbohydrates

Fehling's test: 0.2gm filtrate is boiled on water bath with 0.2ml each of Fehling solutions A and B. A red precipitate indicates the presence of sugar.

Detection of Oils and Resins

Spot test: Test solution was applied on filter paper. It develops a transparent appearance on the filter paper. It indicates the presence of oils and resins.

Antibacterial activity

Preparation of bacterial inoculums

Stock cultures were maintained at $4^{o}C$ on slopes of nutrient agar. Active cultures for experiment were prepared by transferring a loop full of cells from the stock cultures to test tube of Muller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 hrs at $37^{o}C$. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to 2.0×10^{6} colony forming units (CFU/ml) for bacteria.

Bacterial susceptibility test by disc diffusion assay

The disc diffusion method (Bauer *et al.*, 1966) was used to screen the antimicrobial activity. *In vitro* antimicrobial activity was screened by using Muller Hinton Agar (MHA) obtained

from Hi-media (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculums suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. Different concentrations of hydroalcoholic extract of (Leaves) *S.erianthum viz.*, 10, 20, 30 and 40 µg/ml were loaded on 6 mm sterile discs. The loaded discs were placed on the surface of medium and the hydroalcoholic extract of (Leaves) *S.erianthum* were allowed to diffuse for 5 minutes and the plates were kept for incubation at 37^{o} C for 24 hrs. Standard antibiotic disc containing chloramphenicol (1µg/ml) was used as positive control. At the end of incubation zone of inhibition formed around the discs were measured with transparent ruler in millimeter.

Antifungal activity - Preparation of fungal inoculums

The fungal strains were inoculated separately in Sabouraud's dextrose broth for 6 hours and the suspensions were checked to provide approximately 10^5 CFU/ml.

Fungal susceptibility test by disc diffusion assay

All the tests were performed according to Esteban *et al*., 2005. The inoculums was evenly spread on the surface of 10cm petridishes containing Sabouraud's dextrose agar medium (Merck, Germany) and exposed to dry. Standard antibiotic (Fluconazole, concentration 1mg/ml) was used as positive control. Then, the antifungal discs were kept in the plates, after which the plates were incubated at 37^{o}C for 72 hours. After the colonies grew, the diameters of zone of inhibition observed were measured.

RESULTS AND DISCUSSION

Phytochemical Activity

Table 1: Qualitative phytochemical analysis of *S. erianthum.*

S.No	Organism Name	C	10 µg	20 µg	30µg	40 µg
1	*Magnaporthe grisea*	12	3	7	11	16
2	*Botrytis cinera*	14	-	9	14	21
3	*Scopulariopsis Sps*	18	5	11	13	14
4	*Epidermophyton floccosum*	26	-	7	16	18
5	*Aspergillus niger*	17	8	10	13	16
6	*Trichophyton mentagrophytes*	18	3	8	11	14

The phytochemical analysis was carried out for the solvents, Acetone, ethyl acetate and hydroalcohol were used as extractants. The results of phytochemical analysis showed that the leaf of *S.erianthum* has different classes of bioactive constituents. Among the extractants used hydroalcolic extract alone showed the presence of flavonoids, total phenol, tannins, terpenoids and carbohydrate. Which are absent in other two extracts. Steroids in all three extracts and alkaloids in acetone and the presences of alkaloids were noticed in acetone and hydroalcoholic extract.

Table 2: Antibacterial activity of *S. erianthum*.

Phytochemicals	Extracts		
	Acetone	**Ethyl acetate**	**Hydroalcohol**
Alkaloids Mayer's test	+	-	+
Flavonoids H_2SO_4 test	-	-	+
Steroids Liebermann-Burchard test	+	+	+
Terpenoids Salkowski's test	-	-	+
Phenols Ferric chloride test Lead acetate test	- -	- -	+ +
Saponin	-	-	-
Tannin	-	-	+
Carbohydrates	-	-	+
(+) = Detected; (-) = Not Detected			

Antibacterial activity in *S. erianthum* was conducted for six organisms. The highest performance is seen in *Bacillus cereus* with 40 µg concentrations and is higher than the control. For all the other organisms the highest performance is seen in 40 µg concentration and the values are higher than the control. The performance of 10 µg and 20 µg concentrations are lesser than that of the control.

Table 3: Antifungal activity of *Solanum erianthum*.

S.No	Organism Name	C	10 µg	20 µg	30 µg	40 µg
1	*Bacillus cereus*	16	5	15	16	22
2	*Xantho axonopodis*	11	-	6	14	16
3	*Pseudomonas fluorescens*	14	-	-	13	14
4	*Salmonella typhi*	12	-	6	11	13
5	*Escherichia coli*	10	-	10	13	15
6	*Bacillus substilis*	10	4	8	15	20

Anti-fungal activity in *S. erianthum* was conducted with six organisms. The highest activity is seen in *Botrytis cinera* and the activity is higher the control.

CONCLUSION

Highest yield obtained from *S. erianthum* by hydroalcohol extract. The constituents alkaloids, flavonoids, phenols, steroids, terpenoids tannin and carbohydrates are seen in hydroalcohol. Highest anti-bacterial activity is seen in *Bacillus cereus* with 40 µg concentrations and is more than the control. The highest antifungal activity is seen in *Botrytis cinera* for 40µg concentrations. Hydroalcohol leaf extract of *S. erianthum* activity against these organisms offers an important potential for the development of new agents effective against infections which are difficult to treat.

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