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COMPARATIVE STUDY OF DIFFERENT FLOWERING PLANTS ON THE BASIS OF THEIR ANTIFUNGAL ACTIVITY

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ABSTRACT

The present study was undertaken to compare the antifungal activity among flowering plants to know which plant has their antifungal potential. Agar well diffusion method has been used in order to calculate the inhibition length of aqueous and methanol extracts of different flowering plants i.e. Psidium guajava, Polyalthia longifolia, Datura metel, Callistemon lanceolatus & Justicia procumbens against Ryhizoctonia solani, Fusarium oxysporum and Sclerotinia sclerotiorum. Preparation of aqueous and methanol extracts of leaves of these plants was done. The aqueous extract of Psidium guajav has shown maximum antifungal activity against Rhizoctonia solani with an inhibition length of 20 mm and methanol extract of Datura metel has shown maximum antifungal activity against Sclerotinia sclerotiorum with an inhibition length of 30 mm. The methanol extract of Psidium guajava has shown its antifungal activity against Rhizoctonia solani, Fusarium oxysporum and Sclerotinia sclerotiorum with an inhibition length of 22 mm, 9 mm and 3 mm, respectively. No antifungal activity was found to be present against Sclerotinia sclerotiorum in the aqueous extract of Justicia procumbens, where as 16 mm and 10 mm inhibition length was found to be effective against Rhizoctonia solani and Fusarium oxysporum. It can be concluded from above study that the plant Psidium guajava and Datura mete can be utilized as a good source of herbal drugs for various fungal diseases. Future works require the screening of secondary metabolites that are present in these plants.

Keywords: Antifungal Activity, Agar Well Diffusion Method, Flowering Plants

I. INTRODUCTION

Human beings are using medicinal plants as herb in universal phenomena. In the past decades medicinal plant has shown various therapeutic impacts [1]. Herbs and human have shown great relationship with each other. These medicinal plants are known to be used for management of diseases. As far as the traditional medicines are concerned, plant based therapy used as a vital component and serves as a good source for several pharmaceutical drugs used against various diseases [2]. As recognized by WHO that medicinal plants have played an important role in health care of about 80% of world population in countries that are being developed and are depended on tradition medicine. It is also been also stated in previous studies that each and every part of plant has its own antimicrobial activity and also has different secondary metabolites present in them which are responsible for such activities. There are various secondary metabolites in plants which are responsible for

numerous activities such as anti-inflammatory, anti-cancer, anti-fungal, anti-bacterial and others [3]. Flowering plants are referred as angiosperms, because of their reproductive organ such as the flowers which help and explain the success of clades [4]. These flowering plants were more attractive to pollinators and produce more seeds which were attractive to disseminator animals [5].

Psidium guajava commonly known as guava and belongs to family of Myrtaceae. It is a small tree which grows up to 20 ft in height. Its leaves are three to seven inches in length. Flowers of guava are of white color and approx one inch in diameter. Its fruits are in ovoid to pear shape. It is acclaimed to be as the poor man's apple of the tropic [6]. Paste of leaves used to maintain oral hygiene and has shown antimicrobial activity against both gram positive and gram negative bacteria [7]. Its leaves also possess anti inflammatory response by decreasing the CRP levels [8, 9]. *Polyalthia longifolia* commonly known as budha tree or ashoka, belongs to family of Annonaceae. It is an indigenous plant which is native to India. It grows over 30 ft in height. This is evergreen plant, used for the treatment of fever, skin diseases, diabetes and hypertension. It has aromatic leaves which are used for decoration and its barks is used as folk medicine for the treatment of bleeding disorders [10, 11]. Silver nanoparticle has shown effective antibacterial activity against bacterial pathogens [12]. Antifungal activity has also been seen in different solvents of extracts but highly inhibitory effects were observed in petroleum ether extract of leaves [13, 14]. *Datura metel* is also known as thorn apple which belongs to the family of solanaceae. It is native to Marathawada. These are woody, stalked leafy annuals and short lived perennials about 2 m in height. Its leaves are alternate and are toothed margin. Fruit is spiny capsule. The phytoconstituents are tropane alkaloid. It has antimicrobial activity against pathogenic bacteria [15]. *Callistemon lanceolatus* is also known as crimson bottle brush that belongs to family of Myrtaceae. It is indigenous to Queensland and New South Wales. It is small tree height up to 7 cm and leaves are lanceolate shaped. Flowers masses are stamens and pollen at the tip of filaments. Flowers color of *Callistemon lanceolatus* varies species to species that they may be red but some are yellow, green, orange or white and that produce a seed capsules around a stem. It leaves consist of several flavonoids, terpenoids, tannins and phenolic compound. Flowers are rich in polyphenols gallic acid. Essential oils from leaves have shown antifungal activities against *Phaeoramularia angolensis* and *Fusarium oxysporum* [16]. *Justicia procumbens* is also known as water willow and belongs to Acanthaceae family. It is distributed throughout the tropical regions of the world and poorly represented in temperate regions. They are perennial herbs. These species can be easily recognized by bilabial corolla consisting of posterior lip with two lobed, an interior lip is three lobed, two stamens and capsule with four seeds. Methanol extracts of *Justicia procumbens* has shown inhibitory effect against P-388 lymphocytic leukemia growth and in vitro cytotoxicity in the 9-KB (human nasopharyngeal carcinoma) cell culture assay [17]. It exhibited 50% of inhibitory activity with respect to the arachidonic acid-induced aggregation of rabbit platelets [17, 18]. The purpose of present study was to investigate the comparative antifungal activities among these plants by antifungal activities by antimicrobial sensitive test using agar well diffusion method against several fungal strains i.e. *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Fusarium oxysporum*.

II. MATERIALS AND METHOD

2.1 Plant Samples

The leaves of plants (*Psidium guajava*, *Polyalthia longifolia*, *Datura metel*, *Callistemon lanceolatus* and *Justicia procumbens*) were collected from Rajdhani Nursery, Jor Bagh, and New Delhi and used as a plant sample throughout the project.

2.2 Fungal Strains

The fungal strains of *Ryhizoctonia solani*, *Fusarium oxysporum* and *Sclerotinia sclerotiorum* were obtained from Department of Microbiology, IARI, and New Delhi. Isolates of fungi were maintained on the Potato dextrose agar (PDA) slants and stored at 4 $^{\circ}$C at Helix BioGenesis Pvt. Ltd., Noida, U.P. for further use.

2.3 Preparation of Antimicrobial Extracts

2.3.1 Aqueous Extract

Leaves were washed, dried and crushed by pestle and mortal to make fine powder. The two gram of leaf powder was dissolved in 20 ml of distilled water and it was boiled in water bath for 30 min at 100 °C. The conical flasks of extract were covered by cotton plugs to avoid the evaporation. The extracts were placed in shaking incubator at 250 rpm for 48 hrs. After incubation, they were filtered with muslin clothes and again filtered with filter paper twice. The filtered extracts were stored at 4 °C [3, 19].

2.3.2 Methanolic Extracts

Leaves were washed, dried and crushed by pestle and mortal to make fine powder. The two gram of leaf powder was dissolved in 20 ml of methanol and its crude extracts were prepared by maceration method and equally by mixing in organic solvent by boiling at 65 °C. The conical flasks of extract were covered by cotton plugs to avoid the evaporation. The extracts were placed in shaking incubator at 250 rpm for 48 hrs. After incubation, they were filtered with muslin cloth and again filtered with filter paper twice. Methanol was evaporated to dryness and extract amount of extract were measured [3, 19].

2.4 Antimicrobial Sensitive Test Agar Well Diffusion Method

Potato dextrose agar media were prepared, autoclaved at 121 °C for 15 min at 15 Lbs and poured in sterile petri plates up to a uniform thickness of approximately 10 – 15 min, the agar was allowed to set at ambient temperature. This method is suitable for organism to grow rapidly overnight at 35 – 37 °C. The wells were made in medium after inoculation with microorganism 200 µl of inoculums were spread over potato dextrose agar plates using sterile spreader, after few min four wells were made in each petri plate and loaded with 100 µl of plant extracts and control solution (Kirby Bauer method). Plates were incubated at 37 °C for 24 hrs. Antimicrobial activity was observed by measuring inhibition length. The experiments were done in quadruplicates.

III. RESULT AND DISCUSION

In the present study, comparative study was observed between various flowering plants to know which plant has maximum antifungal activity. It was observed from the above study that the aqueous extract of *Psidium guajava* has shown maximum antifungal activity against *Rhizoctonia solani* with an inhibition length of 20 mm as

indicated from the Graph 1 and as compared to previous report relative inhibition found to be present at 1000 ppm with an inhibition length of 1.5 mm [20]. Where as it is also found to be effective against *Fusarium oxysporum* and *Sclerotinia sclerotiorum* with an inhibition length of 9 mm and 4 mm respectively and according to previous studies 45.40 mm pathogen mean/ colony diameter was found to be effective against *Fusarium oxysporum* [21]. Aqueous extract of *Polyalthia longifolia* has shown maximum antifungal activity against *Fusarium oxysporum* with an inhibition length of 18 mm. It has also shown its antifungal activity against *Rhizoctonia solani* and *Sclerotinia sclerotiorum* with an inhibition length of 11 mm and 9 mm as compared to the previous studies which has shown 9 mm of inhibition against *Fusarium oxysporum* [22] and found to be suppressed the formation of *Sclerotinia sclerotiorum* growth at 25% [23]. Antifungal activity was found to be present in aqueous extract of *Datura metel* against *Sclerotinia sclerotiorum* that is 17 mm inhibition length with respect to the previous report, 26.92% of inhibition was observed against *Fusarium oxysporum* [15]. *Callistemon lanceolatus*'s aqueous extract has shown effective antifungal activity against *Fusarium oxysporum* with an inhibition length of 13 mm then *Rhizoctonia solani* & *Sclerotinia sclerotiorum* with an inhibition length of 6mm each, whereas it has caused more than 50% mycelia inhibition of fungi except *Fusarium oxysporum* [16]. No antifungal activity was found against *Sclerotinia sclerotiorum* in the aqueous extract of *Justicia procumbens*, where as 16 mm and 10 mm inhibition length was found against *Rhizoctonia solani* and *Fusarium oxysporum*. The maximum antifungal activity in methanol extract was found to be active in leaves of *Datura metel* against *Sclerotinia sclerotiorum* with an inhibition length of 30 mm and inhibition length of 9 mm against *Rhizoctonia solani* as observed from the Graph 2. The methanol extract of *Psidium guajava* has shown its antifungal activity against *Rhizoctonia solani*, *Fusarium oxysporum* and *Sclerotinia sclerotiorum* with an inhibition length of 22 mm, 9 mm and 3 mm, respectively. In comparison to previous studies, 100% of inhibition was reported found in ethanol extract against *Fusarium oxysporum* sp and 87.5% of inhibition in acetone extract against the same species [24]. Methanol extract of *Polyalthia longifolia* has also shown its comparative effect against all three fungal strains with an inhibition length of 22 mm against *Sclerotinia sclerotiorum* and 16 mm against *Rhizoctonia solani*. As comparative to the previous studies 76% mycelia inhibition observed in 1000 ppm of methanol extract [23]. The methanol extract of *Callistemon lanceolatus* has shown antifungal activity against *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* with an inhibition length of 13 mm, 12 mm, and 2 mm, respectively as comparative to the previous one [16]. No antifungal activity was observed in methanol extract of *Justicia procumbens*.

Table 1: Antifungal Activity of Aqueous Extract of Leaves of Psidium Guajava, Polyalthia Longifolia, Datura Metel, Callistemon Lanceolatus And Justicia Procumbens Against Rhizoctonia Solani

Flowering plant	Well diameter (mm)	Zone of inhibition (mm)	Inhibition length (mm)
Psidium guajava	9	29	20
Polyalthia longifolia	9	20	11
Datura metel	9	0	0
Callistemon lanceolatus	9	15	6
Justicia procumbens	9	25	16

Table 2: Antifungal Activity of Aqueous Extract of Leaves of Psidium Guajava, Polyalthia Longifolia, Datura Metel, Callistemon Lanceolatus and Justicia Procumbens Against Sclerotinia Sclerotiorum

Flowering plant	Well diameter (mm)	Zone of inhibition (mm)	Inhibition length (mm)
Psidium guajava	9	13	4
Polyalthia longifolia	9	18	9
Datura metel	9	26	17
Callistemon lanceolatus	9	15	6
Justicia procumbens	9	0	0

Table 3: Antifungal Activity of Aqueous Extract of Leaves of Psidium Guajava, Polyalthia Longifolia, Datura Metel, Callistemon Lanceolatus and Justicia Procumbens Against Fusarium Oxysporum.

Flowering plant	Well diameter (mm)	Zone of inhibition (mm)	Inhibition length (mm)
Psidium guajava	9	18	9
Polyalthia longifolia	9	27	18
Datura metel	9	0	0
Callistemon lanceolatus	9	22	13
Justicia procumbens	9	19	10

Table 4: Antifungal Activity of Methanolic Extract of Leaves of Psidium Guajava, Polyalthia Longifolia, Datura Metel, Callistemon Lanceolatus and Justicia Procumbens Against Rhizoctonia Solani.

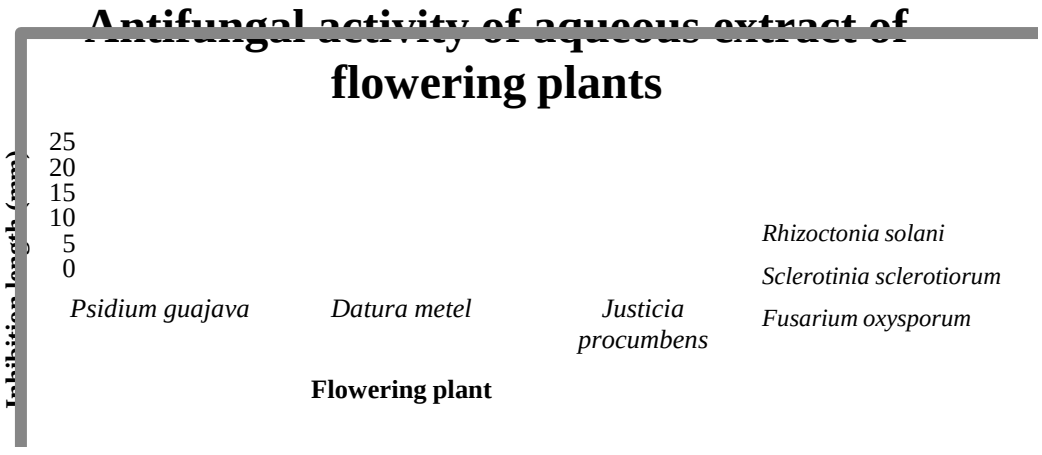
Flowering plant	Well diameter (mm)	Zone of inhibition (mm)	Inhibition length (mm)
Psidium guajava	9	31	22
Polyalthia longifolia	9	25	16
Datura metel	9	18	9
Callistemon lanceolatus	9	21	12
Justicia procumbens	9	0	0

Table 5: Antifungal Activity of Methanolic Extract of Leaves of Psidium Guajava, Polyalthia Longifolia, Datura Metel, Callistemon Lanceolatus and Justicia Procumbens Against Sclerotinia Sclerotiorum.

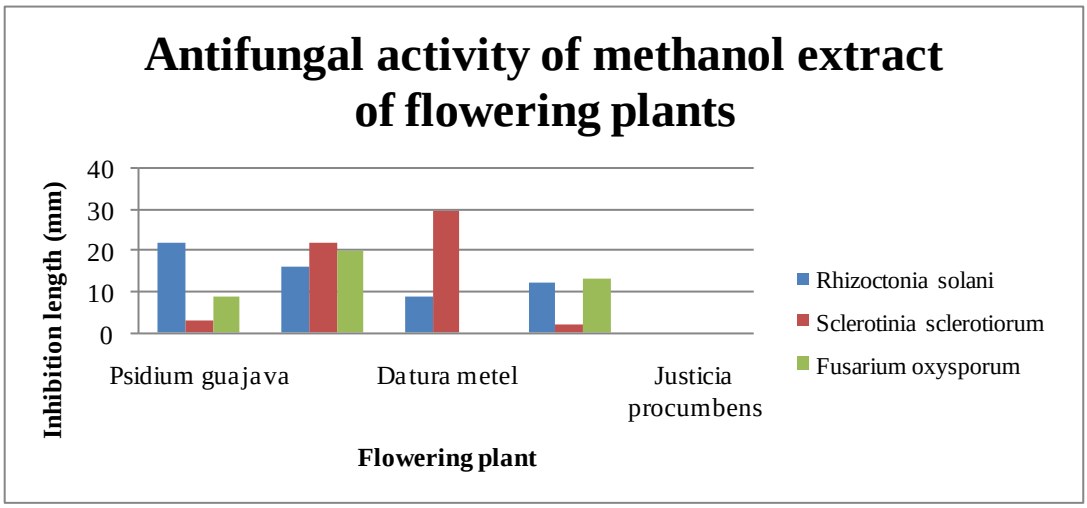
Flowering plant	Well diameter (mm)	Zone of inhibition (mm)	Inhibition length (mm)
Psidium guajava	9	12	3
Polyalthia longifolia	9	31	22
Datura metel	9	39	30
Callistemon lanceolatus	9	11	2
Justicia procumbens	9	0	0

Table 6: Antifungal Activity of Methanolic Extract of Leaves of Psidium Guajava, Polyalthia Longifolia, Datura Metel, Callistemon Lanceolatus and Justicia Procumbens Against Fusarium Oxysporum.

Flowering plant	Well diameter (mm)	Zone of inhibition (mm)	Inhibition length (mm)
Psidium guajava	9	18	9
Polyalthia longifolia	9	29	20
Datura metel	9	0	0
Callistemon lanceolatus	9	22	13
Justicia procumbens	9	0	0



Graph 1: Antifungal Activity of Aqueous Extract of Leaves of Psidium Guajava, Polyalthia Longifolia, Datura Metel, Callistemon Lanceolatus and Justicia Procumbens Against Rhizoctonia Solani, Sclerotinia Sclerotiorum and Fusarium Oxysporum.



Graph 2: Antifungal Activity of Methanol Extract of Leaves of Psidium Guajava, Polyalthia Longifolia, Datura Metel, Callistemon Lanceolatus and Justicia Procumbens Against Rhizoctonia Solani, Sclerotinia Sclerotiorum and Fusarium Oxysporum.

IV. CONCLUSION

It can be concluded from the above studies that among the flowering plants used in the present study, aqueous extract of *Psidium guajava* has shown maximum antifungal activity against *Rhizoctonia solani* and methanol extract of *Datura metel* has shown maximum antifungal activity against *Sclerotinia sclerotiorum*. From the above data, it was observed that the *Datura metel* can be utilized as a good source of herbal drugs for various fungal diseases. Future works require the purification and quantification of secondary metabolites that are present in flowering plants.

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