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Evaluation of Antifungal activity of *Polyalthia longifolia* leaves against *Fusarium spp.*

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ABSTRACT

The oldest form of medicinal services known to mankind is herbal medicines. *Polyalthia longifolia*h as significant use in medicinal science. It is one of the best solutions for the control of alleviating noise pollution. The current research focuses to determine antifungal ability of *Polyalthia longifolia* against various clinically important fungal strain (*Fusarium spp.).* The assessment of the antifungal ability of *Polyalthia longifolia* was measured by poison food method and agar well diffusion method. The maximum extractive value of crude extract of *Polyalthia longifolia* plant leaves obtained in 50% alcohol of *Polyalthia longfolia*. Best inhibitory activity was observed in 50% alcohol extract. Result of present study thus indicates that leaf of *Polyalthia longifolia* processes antifungal activity. *Polyalthia longiifolia* contents secondary metabolites i.e. phenols, flavonoids, Alkaloid, Steroid, Volatile oils, Tannins, Carbohydrate etc which may be responsible for its antifungal activity.

KEYWORDS: antifungal, crude, extract, activity, herbal, leave.

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INTRODUCTION

The need to search a new compound with antimicrobial property has hiked due to the side effects and the development of resistance of drugs in pathogens. The best alternative to the extensive and imprudent use of synthetic compounds as antimicrobial agent may be served by herbal products. From the very beginning of the civilization plants are utilized for the mitigation and management of diseases[1,2]. The oldest form of medicinal services known to mankind are herbal medicines.The important sources for the improvement and development of new compounds of pharmaceutical drugs are traditional medicines. Due to significant drawbacks of chemical control of plant diseases use of the plant extracts is also procuring importance in the control and management of the plant diseases. One such plant is *Polyalthia longifolia* belongs to Annonaceace family[3-5]. The plant is also known as fake ashok, Buddha tree, Indian Mast Tree, False Devadaruand in ayurveda known as Devaderi. *P. longifolia* is used as an ornamental plant for streets as it effectively strive against the noise pollution[2]. It is an evergreen tree with the symmetrical growth with a height over 30 ft. its leaves are narrow lancealate with undulate margins. *Polyalthia longifolia* has significant use in medicinal science[1,5]. It is one of the best solutions for the control of alleviating noise pollution[3]. The current research focuses to determine antifungal ability of *Polyalthia longifolia* against various clinically important fungal strain i.e. *Fusarium spp.*

MATERIALS AND METHODS

Collection of Plant Material:

Sample leaves of *Polyalthia longifolia* were collected. The leaves were first disinfected with 0.1% $HgC1_2$ and subsequently washed twice with distilled water. The leaves were a shade dried and mechanically powdered. Dried and powdered plant leaves were used for preparing crude as well as partially purified extract.

Preparation of Plant extract:

Cold extraction method was used to prepare the leaves extract. The plant extraction was done in the following manner:

Cold Extraction:

The process of cold extraction was used to extract the crude by water as well as alcohol.Cold extractwas prepared according to the modified method of Shadorny and Ingroff (1974). In 100 ml of solvent (water/alcohol) 20 gmof plant material which was being dried and powdered was suspended for

48 hrs. The mixture was then filtered and supernatant was evaporated.The residue was dried and used as an extract[9,15].

Percent Extractive value:

Crude extract was dried in vacuum by usinga rotary evaporator. The estimation of the percentage extractive in terms of the dry weight of the plant material was derived by the following formula:

% extractive = Weight of dried extract / Weight of dried plant material x 100

Media-preparation:

1000 ml of Potato Dextrose Agar was prepared and autoclaved at 121°C at 15 psi pressure. Further it is cool at room temperature and for sterility test kept for one day[8].

Isolation, Identification and Maintenance of Pure culture:

Initial cultures of test pathogens were developed on PDA medium and further purification was done by serial dilution on single spore inoculation method. The cultures were maintained on PDA. Medium was prepared by dissolving desired quantity of ingredients in sterile water[6,7]. The test pathogen was inoculated and incubated aerobically in BOD incubator for 3 days at the optimum temperature of 28-30ºC. Pure cultures of the test pathogens were stored on agar slant at 4ºC and regularly subculture with 3 days old culture.

Identification of fungal culture was confirmed on the basis of colony morphology, culture characteristics and microscopic morphology such as conidial morphology, especially septation, shape, size, color.

Antifungal activity

The assessment of the antifungal ability of *Polyalthia longifolia* was measured by poison food method and agar well diffusion method. In the current study the three extraction media i.e. Acetone, Ethanol and Aqueous were used[11]. Data denotes that the outline of inhibition basically depend upon extraction solvent.

Poison food technique:

This technique was given by Grover and Moore, 1962. Evaluation activities of plant extracts on mycelia growth of *Fusarium spp.*, desired concentrationswere obtained by adding an appropriate

standard solution of plant extract to PDA medium[14]. Each Petriplate was replicated two times and PDA without plant extracts served as control. Each plate was inoculated with 6 mm mycelia disc taken from 3 days old culture of test pathogen on PDA medium and were incubated for 3 days at 37° C. The diameter of colony was measure in each case and percent inhibition was calculated as per formula given by bliss (1934)[13]. The plant extracts which showed maximum antifungal activity against fungus were screened out and evaluated for soil inoculation techniques *in vivo*.

Agar well diffusion:

Molten agar was poured in sterilized Petri plate and allowed to solidify. It was subsequently seeded with the test organism by spreading it on the surface with a sterile spreader then 10 mm wide well was bored in this agar plate and filled with 250 µl of the stock solution of extract (10 mg/ml)[13,14]. The zone of inhibition was measured after 3 days of incubation of the plates at 25° C - 30° C . The method was used for the sensitivity of test fungi against standard drugs with Mancozeb and Bavistin.

Inoculum -Disc:

Three days old culture of the test fungus was used to prepare inoculums discs (6 mm diameter). A single disc was aseptically placed upside down in the center of each Petri plate containing PDA, so as to establish direct contact with the medium[8].

Percentage mycelial growth Inhibition

$$= \frac{P - Q}{Q} \times 100$$

Where,

P = Micelial growth in control after incubation subtracting the diameter of inoculums disc;

Q= Growth of mycelia colony after incubation in treatment set subtracting the diameter of inoculum disc.

OBSERVATIONS

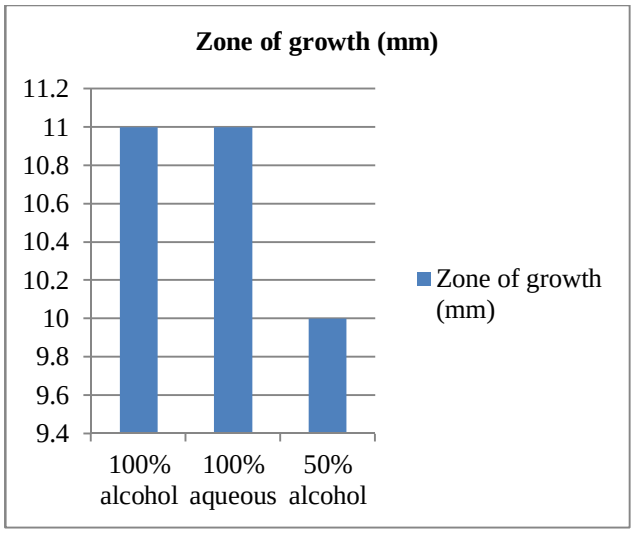

Fig 1: Percentage extractive value of *P. longifolia*

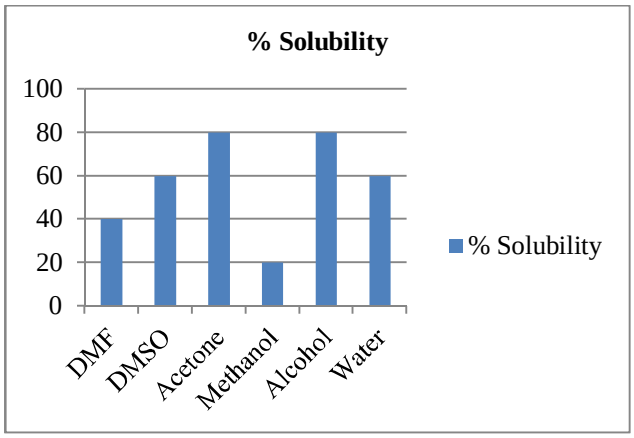

Fig 2: Solvent solubility of100% alcoholic crude extract of *P. longifolia*

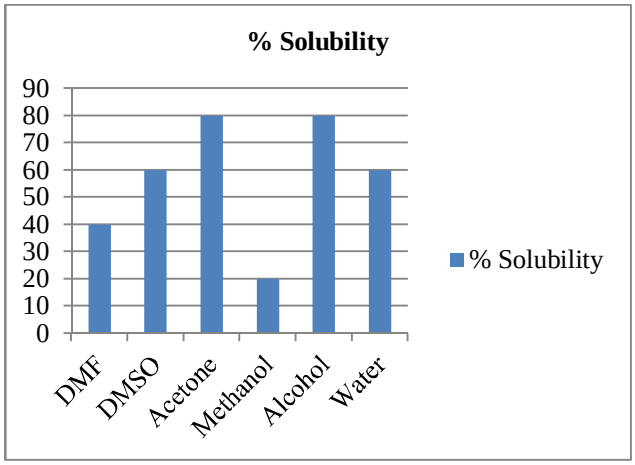

Fig 3: Solvent solubility of 100% aqueous crude extract of *P. longifolia*

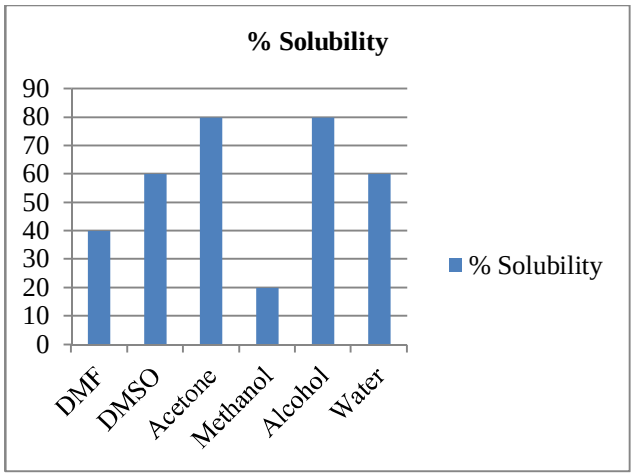

Fig 4: Solvent solubility of 50% alcoholic crude extract of *P. longifolia*

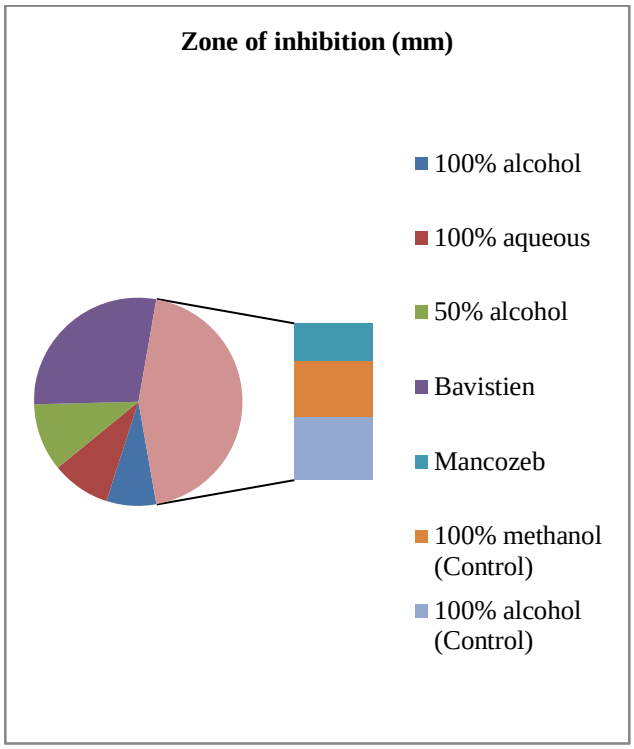

Fig 5: Antifungal activity of *P. longifolia*extracts by Agar well diffusion method

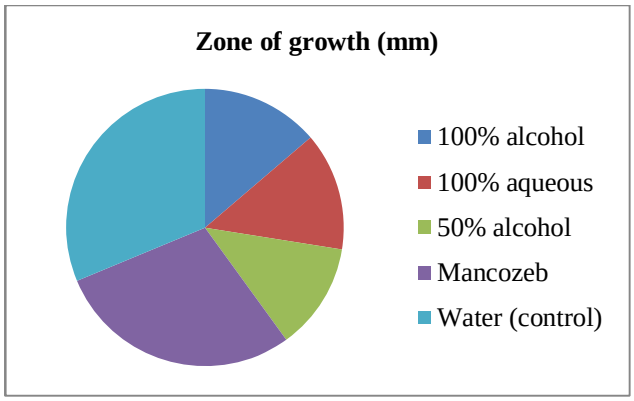

Fig 6: Antifungal activity of *P. longifolia* extract and fungicide byPoisonfood technique

Agar disc diffusion result:

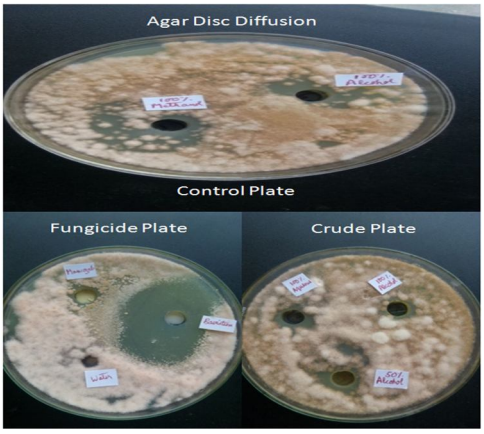

Fig 7: Agar disc diffusion plates

Poison Food Method:

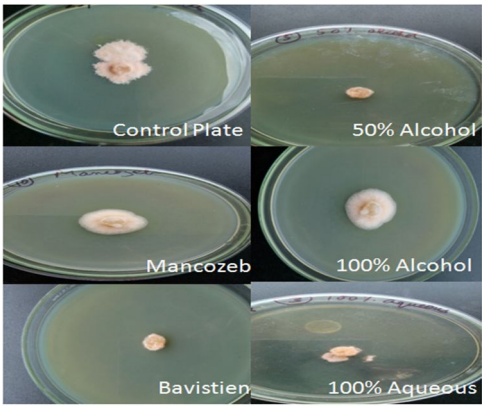

Fig 8: Poison food plates

RESULTS

The maximum extractive value of crude extract of *Polyalthialongifolia* plant leaves was obtained in 50% alcohol of *Polyalthialongfolia* (Fig 2). The solubility test of all three crude in various solvent showed that the extract of 100% alcohol is highly soluble in methanol whereas extracts of 100% aqueous and 50% alcohol is highly soluble in absolute alcohol (Fig 3,4,5). Antifungal activity of alcohol and aqueous extract of *Polyalthialongfolia*is presented in fig 7 and fig 8. Present study revealed that the best inhibitory activity was observed in 50% alcohol extract in both agar well diffusion technique and in poison food technique.

DISCUSSION

Different crude extract of species, herbs and other plant materials rich in polyphenolics are becoming increasingly important in food industries due to their antifungal and antioxidant activity. Such plant derived compound can improve shelf life and maintain quality and nutritional value of stored food commodities.

Present study showed antifungal activity of *P. longifolia* Leaf crude extracts against *Fusarium spp*. Alcohol and water are universal solvent for all kinds of polar compound, but all secondary metabolites show preferential solubility in different solvents based on their polarity, therefore to isolate the compounds it is necessary to prepare extracts in different solvents ranging from non-polar to polar and screen each for anti microbial activity. This provides an idea for the identity of active group of compounds.

Different techniques for anti – microbial susceptibility testing were used in present study. The poison food assay was used for the assay of various crude extracts. The process depends on the reticence of microorganism's growth as an indication of activity which is considered as a function of diameter of growth zone. Although diffusion methods are commonly used for preliminary suseptibility testing but these are not very accurate as diffusion problems cause a high degree of interference with these methods. The more accurate method for antimicrobial susceptibility testing is dilution method.

CONCLUSION

Result of present study thus indicates that leaf of *Polyalthia longifolia* processes antifungal activity. *Polyalthia longiifolia* contents secondary metabolites i.e. phenols, flavanoids, Alkaloid, Steroid, Volatile oils, Tannins, Carbohydrate etc which may be responsible for its antifungal activity. A demand for an economically viable and environmentally safe strategy for the control of diseases had amplified

the use of the products based on plants. Chemical methods deteriorate fertility of soil and characteristics, causes pollution.They also enter the food chain and are responsible for many harmful effect caused on human health and the environment. Therefore, focus is shifting towards alternative strategies for control of fungal diseases.Additionally the methods which are well known for the management of disease as crop rotation, planting disease free seed, use of resistant cultivars, biological control etc. are also practiced. Extract of this plants active molecule can be made to use antifungal formulation which will be important for curing of plant disease and not hazardous for human health.

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