

## IN-VITRO STUDIES ON EFFECT OF LEUCAS ASPERA METHANOLIC EXTRACT AGAINST THREE FUNGAL PATHOGENS OF POMEGRANATE

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### ABSTRACT

Pomegranate is widely cultivated fruit crop in india.it is major source of income for people living in arid region of country. In recent years pomegranate per hectare yield is declined due to diseases outbreaks. Three fungal pathogens, *Cercospora*, *Alternaria* and *Colletotrichum* infect pomegranate flower and fruit and reduces crop yield and quality of fruit . *Leucas aspera* is seasonal weed belongs to the family Lamiaceae is locally known as Dudhani. It grows as a weed at high altitudes of Deccan plature during July to September. The healthy plant of *Leucas aspera* was collected and Methanolic extract was prepared. Antifungal activity was tested by using agar well diffusion method. All studied fungi pathogens were found to be susceptible to *Leucas aspera* plant extract. Extract shows 11 mm inhibition zone against *Alternaria* while against *Cercospora* it shows inhibition zone of 7mm and 6mm zone of inhibition against *Colletotriticum*.

**KEYWORDS:** Antifungal, *Leucas*, Pathogens, Pomegranate,

### INTRODUCTION

Research in agriculture is aimed towards increase of productivity and food quality at reduced expenditure and with increased profit (Sanjeev *et al.*, 2011). In the past few years new trends have emerged in the agricultural sector. Due to the manifestation and developments in the fields of sensor networks, robotics, GPS technology, communication systems etc., precision agriculture started emerging (Camargo and Smith, 2009).

Pomegranate (*Punica granatum* L.) is an ancient fruit, belonging to the smallest botanical family Punicaceae. Pomegranate is a good source of carbohydrates and minerals such as calcium, iron and sulphur. It is rich in vitamin-C and citric acid is the most predominant organic acid in pomegranate (Malhotra *et al.*, 1983). The bark of the stem, root and rind of the fruit is used for slimming, control of dysentery, diarrhea and killing tapeworms (Seema *et al.*, 2015 ).Pomegranate is widely cultivated fruit crop in india. It is major source of income for people living in arid region of country. Farmers prefer ambia-bahar for flowering considering the availability of irrigation water and market demand. In recent years pomegranate per hectare yield is declined due to diseases outbreaks.

Fungal infections cause significant loss in many economic crops. Crop losses are estimated to be about 14% worldwide (Agrios, 2005). Various bacteria and fungi infect pomegranate at various stages, these fungi usually infect during July – September due to high humidity in atmosphere. Three fungal species *Cercospora*, *Alternaria* and *Colletotrichum* infect pomegranate flower and fruit and reduces crop yield and quality of fruit .Progression of these fungal diseases cause favorable environment for entry of bacterial diseases such as bacterial blight. *Colletotrichum* is an important pathogen in different crops. (Pasuvaraji *et al.*, 2013).

*Leucas aspera* belongs to the family Lamiaceae is locally known as Dudhani. It has been reported to be distributed throughout the South Asia. It grows as a weed at high altitudes of Deccan plature during July to September. Traditionally, *L. aspera* has been reported to be used for coughs, cold, painful swelling, and in chronic skin eruptions (Kripa *et al.*, 2011). Isolation of several compounds from different parts of *L. aspera* has been reported including a hydroxytetracontan-4- one, aliphatic ketones, nicotine, farnesene, thujene, menthol from leaf volatiles and amyl propionate, isoamyl propionate from flower volatiles, long chain aliphatic compounds, sterols, triterpenes, phenols, flavonoids such as leucasin from extract (Meghashri *et al.*, 2010). The present study is undertaken to study antifungal activity of *Leucas aspera* against three fungal pathogen of pomegranate such as *Cercospora*, *Alternaria* and *Colletotrichum*.

## MATERIALS AND METHODS

### Collection of plant material:

The healthy mature plants of *Leucas aspera* were collected during the month of September from Sangamner and nearby area. The leaves of plant with apical buds were primarily dried in sunlight and finally dried in an oven at 60°C.

### Extraction

The dried plant material were ground to fine powder with a mechanical grinder and stored in a plastic container. 500 g of ground plant leaf was extracted using Soxhlet extractor by using methanol as extraction solvents. The extract was passed through No. 1 Whatman filter paper. The filtrates obtained were concentrated by rotary evaporator and stored in refrigerator at 4°C for further use.

### Fungal strains isolation:-

Fungal strains were isolated from infected fruits from Sangamner area. Infected fruits were brought to laboratory and fungal species were isolated on PDA medium. PDA medium was prepared by using boiled and squashed potato. Colonies were purified by using transfer and retransfer method. Three fungal species *Cercospora* and *Alternaria Colletotrichum* was selected for experimental purpose.

### Antifungal activity by agar well diffusion method:

A well of 10mm diameter was made using a sterile cork borer on PDA medium. Samples were prepared by dissolving 1mg of crude extracts in 1ml of DMSO. Each well was injected with 20µl of sample in 10mm diameter well. The standard well of 10 mm diameter injected with DMSO (20µg/disc) was used as positive control for antifungal activity. Antifungal assay plates were incubated at 28 ± 2°C for 48-72 h. and diameter of the zone of inhibition was measured and tabulated.

## RESULTS AND DISCUSSION

*In vitro* antifungal activity of *Leucas aspera* against fungi infecting pomegranate was carried out by agar well diffusion method. Methanol used as a solvent for extraction method. Studied pathogens were found to be susceptible to methanol extract of *Leucas aspera*. *Leucas aspera* extract shows significant control of three fungi pathogens in *in vitro* condition. Highest inhibition activity of extract was recorded against *Alternaria* by forming 11 mm inhibition zone, while extract shows 7 mm inhibition zone against *cercospora* under *in vitro* condition while *Colletotrichum* found less susceptible to *Leucas aspera* extract with formation of 6 mm inhibition zone.

**Table 1. Antifungal activity of Methanolic extract of *Leucas aspera* against three fungal pathogens of Pomegranate.**

Sr.No	Name of fungal pathogen	Treated with <i>Leucas aspera</i> extract	Control
1	<i>Alternaria</i>	11 mm	00 mm
2	<i>Cercospora</i>	07 mm	00 mm
3	<i>Colletotrichum</i>	06 mm	00 mm

All studied pathogen found to be susceptible against *Leucas aspera*, so farmer can prepare extract of *Leucas aspera* and use as a fungicide against various pomegranate fungal pathogens.

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