Research Article **ISSN: 2349 – 7106**

Asian Journal of Research in Chemistry and Pharmaceutical Sciences

Journal home page: www.ajrcps.com

ANTIFUNGAL POTENTIAL OF ETHANOL EXTRACTS OF *CALOTROPIS PROCERA* AND *CATHARANTHUS ROSEUS* AGAINST FUNGAL PATHOGENS ON *AEGLE MARMELOS*

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ABSTRACT

The *in vitro* antifungal potential of plant extracts prepared in ethanol of *Catharanthus roseus* and *Calotropis procera* were screened against fungal pathogens isolated from *Aegle marmelos*. Plant extracts have been used as an alternative bio-fungicide against commercial fungicides. Disc diffusion methodology has been used to examine the antifungal potential of the selected plant extracts. Leaf and flower extracts of *C. roseus* and *C. procera* have been studied against the fungal pathogens isolated from various parts of *A. marmelos*. *Alternaria* and *Curvularia* were the isolated fungi treated against the bio-fungicides and compared with the commercial fungicide considered as a control. *C. roseus* showed positive results in comparison to *C. procera*. In addition, flower extracts of *C. roseus* showed comparatively positive results than leaves and stem. The bio-fungicides showed a wide scope as an alternative to commercial fungicide for inhibition of fungal growth on *Aegle marmelos*.

KEYWORDS

Antifungal, Bio-fungicides and Plant extracts.

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INTRODUCTON

Nature has provided a potential source of herbal products and a complete depository of traditional remedies to cure diseases concerned with plants, animals, and mankind (Rahman and Parvin, 2014)[1]. Indian medicinal plants including herbs and shrubs play important role in prevention and treatment of various diseases. Plant diseases across globe represent a critical problem and require prime attention to increase the quality and abundance of crops. Medicinal plants contain several active components which are toxic to fungal pathogens. When extracted from the plants and applied on

infested crops, these components are called botanical fungicides or botanicals. *Aegle marmelos* belonging to family Rutaceae is an Indian medicinal plant, with traditional importance for treatment of several diseases. *Aegle marmelos* is used for various therapeutic and curing purposes by humans including treatment of asthma, fractures, healing of wounds, swollen joints, high blood pressure, jaundice, diarrhea, typhoid, pregnancy complications, and anemia (Sharma *et al.* 2013)[2]. Standardized methods of extractions and *in vitro* antimicrobial efficacy has been tested to explore active plant products as an alternative to synthetic fungicides. Alkhail (2005)[3] studied the antifungal activity of five plant extracts including *Allum sativum*, *Cymogopogon proxims*, *Carumcarvi*, *Azadirachta indica*, and *Eugenia caryophallus*. Similarly, Saini *et al.* (2018)[4], studied the antifungal activity of leaf extracts obtained from medicinal plants against the fungal pathogens found on vegetable beans.

Catharanthus roseus is a tropical plant used for medicinal purpose. The plant contains 2 types of active compound such as alkaloid and tannins. The plant contains resperine. The plant has a long history of use as medicine in Ayurveda and Unani. *Calotropis procera* as a medicinal plant was studied by ancient Egyptians (Mossa *et al.* 1991[5], Greiss 1955)[6]. Extracts from the flowers of *Calotropis procera* have shown strong cytotoxic activity.

Research on bio-fungicides has been conducted to prepare an alternative potential strategy against harmful fungal diseases and pathogens in comparison to commercial fungicides. The study has focused on the ethanol extracts of selected medicinal plants against the fungal pathogens isolated from plant pats of *Aegle marmelos*.

MATERIAL AND METHODS

Plant materials

The plant parts of *Aegle marmelos* were collected randomly from their natural habitat from Ajmer city, Rajasthan, India in the month of September-October 2016. The infected plant parts of *Aegle marmelos* were subjected to isolation and identification of fungi in Potato dextrose agar medium. The isolated fungi were identified according to their macro and microscopic structures.

Preparation of plant extracts

The extracts were prepared from stem, leaves and flowers of *C. roseus* and *C. procera*. Fresh plant parts were washed thorough tap water followed by alcohol and autoclaved distilled water. After the drying process, the plant parts were crushed with the help of pestle and mortar. Ethanol was used for preparation of plant extraction. The plant materials were subjected to centrifugation for 10-15 min (at 10000 rpm). The supernatant was collected after filtration and made to known volume by adding sterile ethanol stored for further antimicrobial screening purpose.

Antifungal activity

The antifungal activity for the selected plant extracts were studied by disc diffusion method (Perez *et al.*1990)[7]. Sterile discs of equal size (5mm) were prepared of autoclaved Whatman's filter paper. Infused with different plant extracts overnight. The impregnated disc was considered as suitable for antifungal assay according to the color change of the disc. The Petri dishes were marked with equally distributed chambers at the bottom for placing the disc. The discs were placed on Czapek dox agar media already poured with the test fungal broth. The Petri dishes were incubated for 7 days with regular observation every day. Similar protocol was followed for the commercially selected fungicide fluconazole. After the selected time period, zone of inhibition in mm was measured by measuring scale and pictures were taken. A comparative study of both plant extracts and commercial fungicide was subjected for antifungal bioassay.

RESULTS AND DISCUSSION

Antifungal activity of two medicinal plant extract prepared in ethanol was studied by disc diffusion method. The results obtained showed positive to neutral effect on the growth inhibition of fungal pathogens isolated from *A. marmelos* plant parts. Table No.1. Shows the results of different plant extracts treated against the fungal pathogens.

Among the ethanol plant extracts studied, ethanol extract of *Calotropis procera* showed 100% antifungal potential against fungal pathogen *Curvularia*. *Catharanthus roseus* flower and *Calotropis procera* leaves showed positive results with 26 mm and 24 mm diameter zone of inhibition. However, ethanol plant extracts of *C. roseus* leaves showed similar results of 6 mm diameter zone of inhibition with fluconazole (control).

Table No.1: Antifungal activity of plant extracts treated against fungal pathogen isolated from Aegle marmelos

S.No	Medicinal plant	Plant part	Solvent	Isolated fungal pathogen	Zone of inhibition (diameter in mm)
1	*Calotropis procera*	Leaves	Ethanol	*Curvularia*	24
2	*Calotropis procera*	Flower	Ethanol	*Curvularia*	No fungal growth
3	*Catharanthus roseus*	Leaves	Ethanol	*Curvularia*	6
4	*Catharanthus roseus*	Flower	Ethanol	*Curvularia*	26
5	*Catharanthus roseus*	Stem	Ethanol	*Curvularia*	22
6	Fluconazole	-	Ethanol	*Curvularia*	6

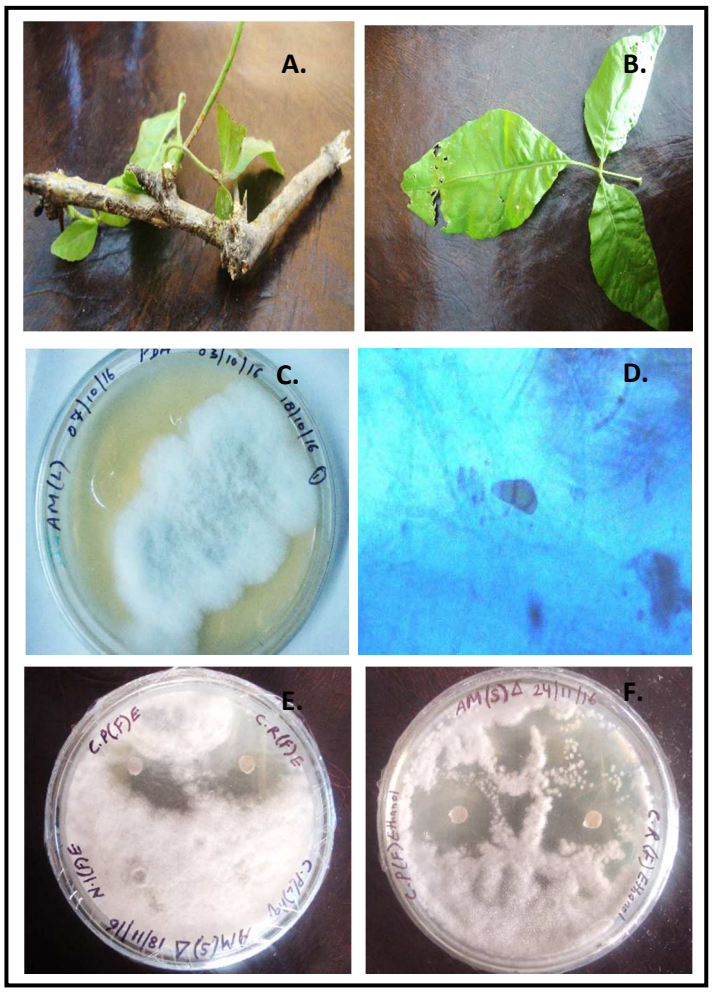

Figure No.1: Study of fungal pathogens and antifungal activity of plant extracts. A, B. Infected plant of *A. marmelos*. C. Pure culture of fungal growth from leaves of *A. marmelos*. D. isolated *Curvularia*. E, F. antifungal activity of plant extracts prepared from *C. procera* and *C. roseus* against fungal pathogens

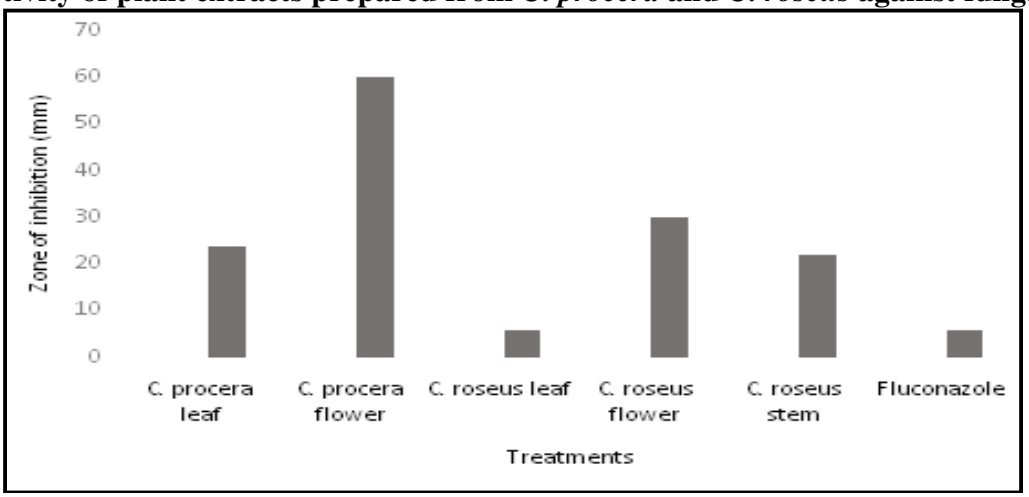

Figure No.2: Comparative graphical representation of different botanical ethanol extracts with commercial fungicide fluconazole against fungal pathogen isolated from *A. marmelos*

CONCLUSION

Bio-fungicides are cheaper and comparatively more effective in inhibiting the growth of the fungal pathogens found on plants. *Aegle marmelos* being an important medicinal plant has been taken as a test plant for isolation and treatment of fungal pathogens.

ACKNOWLEDGEMENT

The authors would like to express sincere thanks to the chair, Department of Botany, S.P.C. Government College, Ajmer, Rajasthan, India for providing the facilities for smooth conduction of this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: Shruti Ojha and Mamta Goyal. Antifungal potential of ethanol extracts of *calotropis procera* and *catharanthus roseus* against fungal pathogens on*aegle marmelos*, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences,* 7(1), 2019, 241-245.