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Original Research Article

Antifungal efficiency of amla leaves against *Alternaria alternata* and *Bipolaris spicifera*

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ABSTRACT

In the present study, *amla leaves* have been investigated for antifungal potential against two phytopathogens namely *Alternaria alternata* and *Bipolaris spicifera*. Inhibitory potential of methanolic extract of the plant was analyzed by poisoned food technique in which different concentrations of test material were prepared in sterilized potato dextrose agar. Results of the investigation revealed that *Embilica officinalis* has potential activity against both the test pathogens with IC_{50} value of 1±0.02 mg/mL and 0.95±0.019 mg/mL against *Alternaria alternata* and *Bipolaris spicifera* respectively. To conclude, leaves of amla possess potential antifungal activity and may be used as a resource of biocides in pesticide and food industry.

1. Introduction

Embilica officinalis known as Indian gooseberry or aonla/amla, belonging to family *Euphorbiaceae*, is a medium- sized deciduous tree with gray bark / reddish wood and is native to Tropical Sourthern Asia, particularly India. Amla fruit is highly nutritious, is one of the richest sources of vitamin-C, amino acids and minerals and contains several chemical constituents like tannins, alkaloids and phenols (Srivasuki et al., 2012; Zhang et al., 2003). A number of chemical constituents from every part of the plant have been extracted and several systematic scientific studies are also being conducted regarding the efficacy of whole plant or its parts in different extract forms for the treatment of different diseases. (Sharma et al., 2009). Amla extracts revealed antibacterial / antifungal (Hussain et al., 2012), antioxidant (Golechha et al., 2012), cardio-protective (Bhattacharya et al., 2002), anthelmintic (Dwivedi et al., 2011) and anti-inflammatory (Mishra et al., 2004) properties. Pharmacological research reports on amla reveals its analgesic (Sharma et al., 2004), anti-tussive (Nosal et al., 2003), anti-atherogenic (Santosh et al ., 2013), adaptogenic (Muruganandam et al., 2002), cardio (Baliga et al., 2013), gastro (Chatterjee et al., 2011), nephro (Yokozawa et al ., 2007), neuro-protective (Vasudevan et al., 2007) and anticancer (Madhuri et al., 2008) properties. Amla is also reported to possess chemopreventive (Krishnaveni et al., 2012) radio (Adil et al., 2010), chemo (Deep et al., 2005) and immunomodulatory (Varadacharyulu et al., 2009), free radical scavenging (Prakash et al., 2012) antioxidant, (Nripendranath et al., 2010), anti-inflammatory, (Santoshkumar et al., 2013) anti-mutagenic activities. Fungal diseases are a major cause of mobidity and mortality worldwide and pathogenic fungi are the main infectious agents in plants causing alterations during developmental stages including post-harvest.

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Table 1: IC_{50} values of amla extract against two crop infecting fungi

Pathogen [a]	Antifungal activity IC50 (mg/mL) MeOH
Bipolaris spicifera	0.95±0.019
Alternaria alternata	1± 0.02

[a] Data presented is mean ± s.d. of three replicates.

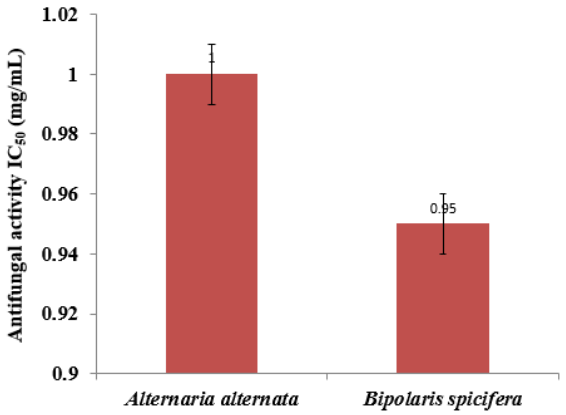

Figure 1: Antifungal activity of amla leaves against *Alternaria alternata* and *Bipolaris spicifera*

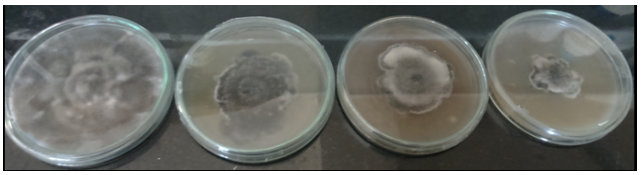

Figure 2: Antifungal activity against *Bipolaris spicifera*

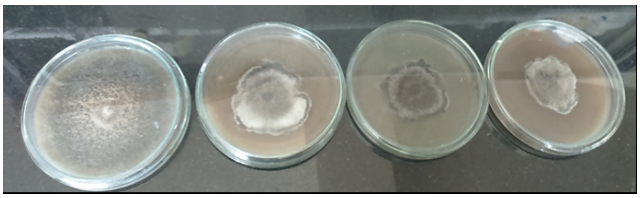

Figure 3: Antifungal activity against *Alternaria alternata*

2. Materials and Methods

2.1. Preparation of extracts

Fresh leaves of amla were identified and collected from Herbal Garden, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu (SKUAST-J). The freshly collected leaves were shade dried and ground into powdered form. The powdered dried leaf material was then extracted with organic solvent at room temperature to obtain extract for bioevaluation. The methanolic extract was prepared by percolating the dried ground plant material (100 g) with 99 per cent methanol that was evaporated using rotary vacuum evaporator.[1–8]

2.2. Determination of antifungal activity by poisoned food technique

Different concentrations of test component (extract) were prepared in sterilized potato dextrose agar and poured in 9 cm petri plates. After this, 5 mm bit of test fungus was inoculated in the center of the agar plate (mycelia surface of the bit was placed upside down) followed by incubation of petri plates at 26 ^{0}C. The extension diameter (mm) of hyphae from the center to the dish was measured at 24 h interval, till the growth of fungus in the plate without test component (control) reached the edge of the plates. The experiment was repeated thrice and results were expressed as average of three replicates (Guleria et al., 2013)

Fungal growth diameter in each plate containing concentrations of test component was determined to calculate per cent growth inhibition.

The antifungal indices was calculated as:

Antifungal index (%) = (1-D_a/D_b) ×100

D_a = Diameter of growth zone in the experiment dish (mm)

D_b = Diameter of growth zone in the control (mm)

3. Results and Discussion

To evaluate the antifungal activity of methanolic extract from amla leaves, two agriculturally important phytopathogenic fungi - *Alternaria alternate* and *Bipolaris spicifera* were selected. Results in the present study revealed that the amla leaves possess potential antifungal activity against two pathogenic fungal species. It is found that methanolic extract of amla inhibited the growth of the colonies of large number of fungal species. The antimicrobial efficacy of extract of amla was qualitatively assessed on the basis of inhibition zone. In literature, many medicinal plants indicated their strength through antimicrobial behavior that was endorsed with high concentration of flavonoids and alkaloids.[9–12]

The results of the present study showed that the antifungal activity of *amla* against both the test pathogens with IC_{50} values were 1±0.02 and 0.96±0.019 mg/mL respectively. The search for antimicrobials from natural sources has received much attention and efforts have been put in to identify compounds that can act as suitable antimicrobials agent to replace synthetic ones. Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of micro-organism. These compounds have significant therapeutic application against human pathogens including bacteria, fungi or virus. Numerous studies have been conducted with the extracts

of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds.[13–16] Therefore, medicinal plants are finding their way into pharmaceuticals, neutralceuticals and food supplements. Further, research has to be conducted to find out the possibility of this medicinally important plant as a potent antimicrobial drug and for other pharmacological properties to develop as cost effective formulation.

4. Conclusion

To conclude, many of the phytochemicals found in this particular plant proved to have potential antimicrobial properties, which has to further investigated. Complete purification and identification of the biologically active compounds might help in understanding the mechanism of action and thereby to formulate a new potent drug.

5. Source of Funding

None.

6. Conflict of Interest

None.

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