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**IN VITRO STUDIES ON ANTIFUNGAL ACTIVITIES OF
PHYLLANTHUS EMBLICA**



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Abstract

The research work was conducted with the fruits of *Emblica officinalis* (Fam: *Euphorbiaceae*) to investigate antifungal activities. The fruits of the plant were successively extracted by cold extraction process by using two solvents namely ethanol and acetone. Antifungal screening was done for the ethanol and acetone extracts by disk diffusion method with five pathogenic fungi. Both the extracts showed moderate activity against *Fusarium equiseti* and *Candida albicans*. In this case, Grisofulvin was used as standard antibiotic.

Keywords: - Phyllanthus Emblica, Grisofulvin

Introduction

Herbal medicines are assuming large use in the primary healthcare of individuals and communities consistently with the growing interest in traditional and alternative system of medicine in many developed countries. Several consumer surveys indicated a positive public attitude to complementary medicines. Herbal medicine as the major remedy in traditional systems, have been used in medical practice and made a great contribution to maintain human health. Herbal medicine is one of several holistic therapies gaining recognition within the health care community worldwide. As a discipline, herbal medicine is in infancy regarding educational standards for credential, standardization and regulation of products and clinical application.

It has now been established that the plants which naturally synthesize and accumulate some secondary metabolites like alkaloids, glycosides, tannins, volatiles oils and contain minerals and vitamins possesses medicinal properties (Abdul Ghani, 1998). Recent trend is to integrate the traditional medicine with modern medicine. Best therapeutic results are said to be obtained often with the traditional Chinese system (Meyer *et al.*, 1982).

Emblica officinalis popularly known as amla, is a deciduous tree having average height of 5.5 metres.

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The fruit is drupe, 1.5-2.5 cm. in diameter, smooth, shiny with light coloured specks. It is distinctly marked in six lobes. The fruit is green when tender but the colour changes to light yellow or brick red on maturity. The taste is sour and astringent giving feeling of sweetness afterwards (Mohammed Ali, 1998). The plant is found in the mixed deciduous forests of India, Sri Lanka, China, Bangladesh and Malaya ascending to 1,500 metres on the hills (Mohammed Ali, 1998). Dried fruit is useful in haemorrhage, diarrhoea, diabetes and dysentery. The fruit has antibacterial, antifungal, and antiviral activities (Mohammed Ali, 1998). This plant has been chosen for the present investigation because of its availability and wider indication in various diseases. Therefore, in present work a humble attempt was made to detect the presence of antifungal activities of this plant's fruits by a simple and commonly used agar diffusion method for investigation.

MATERIALS AND METHODS

Collection: The fruits of the plant Amla or *Emblica officinalis* were collected and then cleaned from impurities. The fruits of the plant were air dried properly for 7 days. After complete drying, the fruit samples were ground into coarse powder with the help of a mechanical grinder and the powder was stored in a suitable container for extraction process.

Preparation of the Plant Extract: The powdered material was successively extracted with ethanol and acetone by using cold extraction process (Trease and Evans 1983). At first 250 gm of dried powder was taken in an aspirator (5L). Before placing powders into the aspirator, the jar was washed properly and dried.

Then 750 ml of solvent ethanol was added gradually. The time duration was of 21 days at room temperature with occasional shaking and stirring for each successive extraction. It was then filtered through a fresh cotton plug and finally with a Whatmann filter paper no. 1. In the same way the powdered material was extracted with acetone. Finally this two extracts were concentrated by rotary evaporator in dry & clean air.

Antifungal Assay: Study of *in vitro* antifungal activities of the ethanol and acetone extracts obtained from the extraction of the fruits of the plant *Emblca officinalis*. Five pathogenic fungi were used as test organisms for antifungal activity of the dried extracts. Both extracts were tested for antifungal study by using standard disc diffusion method (Murray *et al.*, 1995 and Zavalla *et al.*, 1997). Nutrient agar media was used for culture of the test organisms and the antifungal activities were determined by single disc diffusion method (Barry, 1980). Nutrient agar medium (23 gm) was suspended in 1000 ml of water and heated to make a clear solution. Then from this clear solution concentrated agar plates were prepared. The standard discs 0.1mg Grisofulvin/disc was used to compare the both activities of test samples. For extracts 0.5mg/disc samples were used. The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates, pre-inoculated with test organism. The discs were then incubated on the plate aerobically at 37°C for 24 hours. The diameter of inhibition zone around each disc was measured and recorded at the end of the incubation period. The extract concentration able to inhibit microbial growth, which was observed through the formation of an inhibition growth zone around the disc (equal to or greater than 8 mm) (Bauer, 1966) was considered.

RESULTS AND DISCUSSION

In vitro Antifungal Test: The ethanol & acetone extracts of the sample were tested for antifungal activity against five human & phytopathogenic test fungi. Both the solvent extracts showed moderate activity against *Fusarium equiseti* and *Candida albicans* organisms.

The observed antifungal activity, measured in terms of diameter of zone of inhibition in mm are showed in **table 1**.

TABLE 1: *IN VITRO* ANTIFUNGAL ACTIVITY OF THE ETHANOL & ACETONE EXTRACTS OF FRUITS OF *EMBLICA OFFICINALIS*

Test Fungi	Zone of inhibition in diameter (mm)		
	Ethanol extract (0.5mg/disc)	Acetone extract (0.5mg/disc)	Standard (0.1mg/disc)
<i>Alternaria alternate</i>	NS	NS	NS
<i>Botryodiplolia theobromae</i>	NS	NS	9.0
<i>Fusarium equiseti</i>	9.5	10.0	11.0
<i>Aspergillus niger</i>	NS	NS	12.0
<i>Candida albicans</i>	11.0	9.5	11.5

Standard (Grisofulvin solution); NS = Not Susceptible

From table 1, it is found that both extracts showed antifungal activity only against *Fusarium equiseti* and *Candida albicans*. On the other hand, the standard sample (Grisofulvin) exhibited activity against all fungi tested except *Alternaria alternate*.

In this study, we used disk diffusion method and a number of human & phytopathogenic fungi for the determination of antifungal activity of the fruits of plant. Both solvent extracts showed antifungal activity against only two test fungi. In this case, the standard drug sample was Grisofulvin .

CONCLUSION

From thorough study of this experiment it may be concluded that, the extracts of two different solvents of fruits of *Emblca officinalis* has narrow spectrum antifungal property against some pathogenic organism strains in this research work. As evident from the above discussion, fruits of *Emblca officinalis* may contain important chemical substances that confer upon this plant as medicinal agent possessing antifungal activity. As apparent from our results and from other worker's reports, local uses of the fruits of this plant in various diseases are not at much variance with its antimicrobial property. This fact also indicates that the traditional uses of this plant's fruits are not scientifically baseless and therefore, the other plant parts and obviously fruits of *Emblca officinalis* should be thoroughly investigated phyto-chemically to fully exploit its medicinal and pharma-ceutical potentialities.

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