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IN VITRO STUDIES ON ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF *EMBLICA OFFICINALIS*

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ABSTRACT

The research work was conducted with the fruits of *Emblica officinalis* (Fam: *Euphorbiaceae*) to investigate antibacterial & antifungal activities. The fruits of the plant were successively extracted by cold extraction process by using two solvents namely ethanol and acetone. Antimicrobial activities of the extracts of both solvents were investigated by a simple agar diffusion method using ten pathogenic bacteria. The extracts of ethanol showed moderate activity against *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis*, *Shigella dysenteriae* and *Bacillus megaterium*. Again acetone extracts showed moderate activity against *Vibrio cholerae*, *Staphylococcus aureus*, *Bacillus subtilis* and *Shigella dysenteriae*. All the activities were determined by measuring the zone of inhibition compared with the standard antibiotic (Amoxicillin). Antifungal screening was done for the ethanol and acetone extracts by disk diffusion method with ten pothogenic fungi. Both the extracts showed moderate activity against *Fusarium equiseti* and *Candida albicans*. In this case, Grisofulvin was used as standard antibiotic.

INTRODUCTION: The contributions of the plants are enormous in every sector of human life. It helps to growing up of the human body and also protects human being from sickness by being used as medicine. A large number of plants are used as medicinal agents in this world. Specifically in Bangladesh about two hundred fifty species are used as medicinal plants [1]. 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 [2]. 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 [3].

The drug resistance of human and animal pathogens is one of the best documented cases of biological evolution, and is a serious problem both in developed and developing countries. The daily consumption of more than one ton of antibiotics in some countries has resulted in resistance to bacterial populations, thus causing a serious public health problem. In face of this scenario, the search for substances from natural sources, including plants, has been gaining importance in the pharmaceutical companies [4].

Emblica officinalis popularly known as amla, is a deciduous tree having average height of 5.5 metres. The fruit is drupe, fleshy globose, 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 [5]. 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 [6].

Dried fruit is useful in haemorrhage, diarrhea, diabetes and dysentery. The fruit has antibacterial, antifungal, and antiviral activities [7]. 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 antibacterial and antifungal activities of this plant's fruits by a simple and commonly used agar diffusion method for investigation.

MATERIALS AND METHODS:

Identification and Collection: Using standard taxonomical methods, supplied by the Bangladesh Forest Research Institute (BFRI), Chittagong, identified the plant's fruits. The fruits of the plant Amla or *Emblica officinalis* were collected from Baluchora, Chittagong on July, 2011 and was identified by a Taxonomist of Bangladesh National Herbarium (BNH). They were then separated & 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 [8]. 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. Finaly this two extracts were concentrated by rotary evaporator in dry & clean air.

Antibacterial and Antifungal Assay: Study of both *in vitro* antibacterial and antifungal activities of the ethanol and acetone extracts obtained from the extraction of the fruits of the plant *Emblica officinalis*. Ten pathogenic bacteria and fungi were used as test organisms for antibacterial activity of the dried extracts. Both extracts were tested for antimicrobial study by using standard disc diffusion method [9, 10].

The bacterial and fungal strains were collected from the microbiology laboratory of BCSIR, Chittagong. Nutrient agar media was used for culture of the test organisms and the antibacterial and antifungal activities were determined by single disc diffusion method [11].

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 Amoxicillin/disc and 0.1mg Grisofulvin/disc were 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^{o}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) [12] , was considered.

RESULTS AND DISCUSSION:

***In vitro* Antibacterial Study:** The extracts of the sample were tested for antibacterial activity against ten pathogenic both gram-positive and gram-negative bacteria. Standard antibiotic disk of amoxicillin was used for comparison purposes. The two different extracts of fruits of the plant showed moderate antibacterial activity against some of the test organisms. The results of the antibacterial activity, measured in terms of diameter of zone of inhibition in mm are showed in **table 1**. The zone of inhibition was found in different organisms.

From the table 1, it is observed that, the ethanol extract showed antibacterial activity against some test organisms namely- *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis*, *Shigella dysenteriae*, & *Bacillus megaterium*. On the other hand, the acetone extract of fruits of *Emblica officinalis* showed activity against *Vibrio cholerae*, *Staphylococcus aureus*, *Bacillus subtilis* & *Shigella dysenteriae*.

Again the standard sample (Amoxicillin) showed remarkable activity against all tested bacteria except *Salmonella typhi*.

The zone of inhibition found in different organisms can be shown in the following **figure 1**, in mm.

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

Test Bacteria	Zone of inhibition in diameter (mm)		
	Ethanol extract (0.5mg/disc)	Acetone extract (0.5mg/disc)	Standard (0.1mg/disc)
Vibrio cholerae	NS	9.5	10.0
Staphylococcus aureus	10.6	10.0	28.0
Salmonella typhi	9.0	NS	NS
Pseudomonas aeruginosa	NS	NS	34.4
Bacillus subtilis	11.0	10.7	33.0
Bacillus cereus	NS	NS	9.5
Shigella dysenteriae	12.7	11.0	28.0
Staphylococcus pyogenous	NS	NS	35.8
Escherichia coli	NS	NS	38.5
Bacillus megaterium	8.5	NS	26.3

Standard (Amoxicillin solution); NS = Not Susceptible

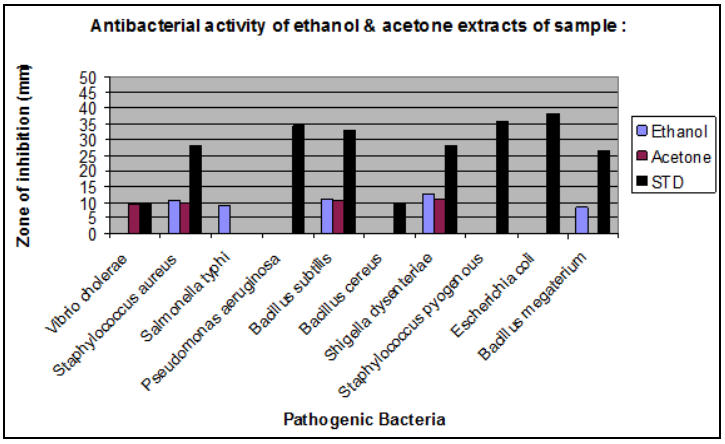

FIGURE 1: ZONE OF INHIBITION OF THE ETHANOL & ACETONE EXTRACTS OF FRUITS OF *EMBLICA OFFICINALIS*

***In vitro* Antifungal Test:** The ethanol & acetone extracts of the sample were tested for antifungal activity against ten 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 2**.

TABLE 2: *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)
Alternaria alternate	NS	NS	NS
Botryodiplolia theobromae	NS	NS	9.0
Colletotrichum corcori	NS	NS	8.0
Curvularia lunata	NS	NS	NS
Fusarium equiseti	9.5	10.0	11.0
Fusarium solanii	NS	NS	9.5
Aspergillus niger	NS	NS	12.0
Candida albicans	11.0	9.5	11.5
Colletotrichum gleosparoitis	NS	NS	NS
Macrophomina phaseolina	NS	NS	12.5

Standard (Grisofulvin solution); NS = Not Susceptible

From table 2, 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*, *Curvularia lunata* and *Colletotrichum gleosparoitis*. The zone of inhibition found in different organisms can be shown in the following **figure 2**, in mm.

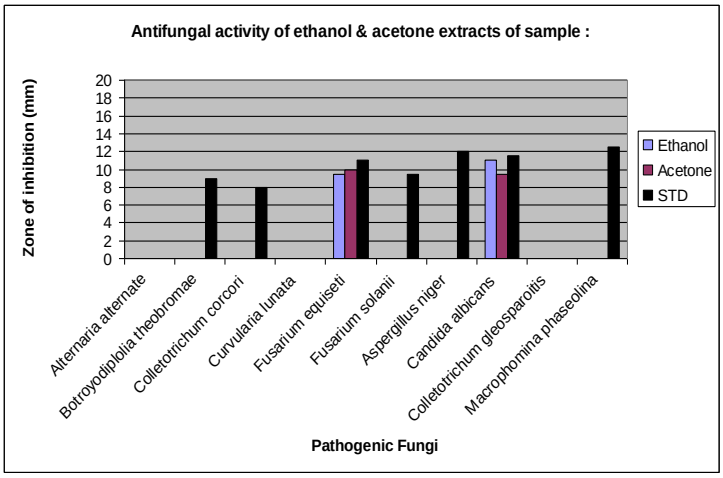

FIGURE 2: ZONE OF INHIBITION OF THE ETHANOL & ACETONE EXTRACTS OF FRUITS OF *EMBLICA OFFICINALIS*

CONCLUSION: *In vitro* antibacterial study of two different solvent extracts of fruits of *Emblica officinalis* was done by simple agar diffusion method. In this experiment ten pathogenic bacteria were used. The ethanol extracts exhibited antibacterial activity against five of the pathogenic organisms and acetone extracts showed activity against four of the bacteria tested. Again 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 both of the cases, the standard drug samples were Amoxicillin and Grisofulvin respectively. From thorough study of this experiment it may be concluded that, the extracts of two different solvents of fruits of *Emblica officinalis* has narrow spectrum antibacterial and antifungal property against some pathogenic organism strains in this research work. As evident from the above discussion, fruits of *Emblica officinalis* may contain important chemical substances that confer upon this plant as medicinal agent possessing antibacterial and 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 *Emblica officinalis* should be thoroughly investigated phyto-chemically to fully exploit its medicinal and pharmaceutical potentialities.

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