IJPSR (2013), Vol. 4, Issue 1 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 30 June, 2012; received in revised form, 07 December, 2012; accepted, 29 December, 2012

IN VITRO EVALUATION OF ANTIBACTERIAL AND ANTIFUNGAL PROPERTIRS OF *TERMINALIA BELERICA*

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Keywords:

Terminalia belerica, Antibacterial activity, Antifungal Activity, Zone of inhibition, Agar diffusion method

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QUICK RESPONSE CODE

IJPSR: ICV- 5.07

Website: www.ijpsr.com

ABSTRACT

The research work was conducted with the fruits of *Terminalia belerica* (Fam: Combretaceae) 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 all tested pathogenic bacteria except *Bacillus subtilis*. Again acetone extracts showed moderate activity against *Vibrio cholerae, Staphylococcus aureus, Bacillus subtilis, Shigella dysenteriae* and *Escherichia coli*. 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 pathogenic fungi. Both the extracts showed moderate activity against *Colletotrichum corcori*, *Fusarium equiseti* and *Candida albicans*. In this case, Griseofulvin was used as standard antibiotic.

INTRODUCTION: *Terminalia belerica* is a perennial herb mainly distributed in the tropical regions and commonly found in South-East Asia, including Thailand. It is one of the ingredients of "tripala", an Ayurvedic formulation that is believed to promote health, immunity and longevity [1]. This formulation, rich in antioxidants, is frequently used in Ayurvedic medicine to treat many diseases such as anemia, jaundice, constipation, asthma, fever and chronic ulcers [2].

The fruit of *T. belerica* has been used to treat various ailments in the folklore medicine [3]. Antibacterial [4], antidiabetic, and antioxidant [5] activities of crude extracts of *T. belerica* have been reported. The fruit is also reported to have purgative [6], cardiac depressant, hypotensive and chleretic effects [7].

Chemically, the presence of sitosterol, gallic acid, ellagic acid, ethyl gallate, chebulagic acid, mannitol, glucose, galactose, rhamnose, and fructose have been reported in the fruit of *T. belerica* [8].

MATERIALS & 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 *Terminalia belerica* were collected from Baluchora, Chittagong on August 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 [9]. 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 *T. belerica*. 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 [10, 11]. 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 [12].

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 37ºC 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) [13] 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.

TABLE 1: *IN VITRO* ANTIBACTERIAL ACTIVITY OF THE ETHANOL & ACETONE EXTRACTS OF FRUITS OF *TERMINALIA BELERICA*

Test Bacteria	Zone of inhibition in diameter (mm)_		
	Ethanol extract (0.5mg/disc)	Acetone extract (0.5mg/disc)	Standard (0.1mg/disc)
Vibrio cholerae	11.5	9.0	12.0
Staphylococcus aureus	14.0	12.5	20.0
Salmonella typhi	9.0	NS	10.0
Pseudomonas aeruginosa	8.0	NS	28.5
Bacillus subtilis	NS	15.0	38.0
Bacillus cereus	13.5	NS	12.0
Shigella dysenteriae	12.7	11.0	28.0
Staphylococcus pyogenous	10.5	NS	16.5
Escherichia coli	15.0	10.4	21.0
Bacillus megaterium	12.0	NS	20.5

Standard (Amoxicillin solution); NS = Not Susceptible

From the table 1, it is observed that, the ethanol extracts showed antibacterial activity against some test organisms namely- *Vibrio cholerae, Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Bacillus cereus, Shigella dysenteriae, Staphylococcus pyogenous, Escherichia coli* and *Bacillus megaterium*. On the other hand, the acetone extracts of fruits showed activity against *Vibrio cholerae, Staphylococcus aureus, Bacillus subtilis, Shigella dysenteriae* and *Escherichia coli*. Again the standard sample (Amoxicillin) showed remarkable activity against all tested bacteria.

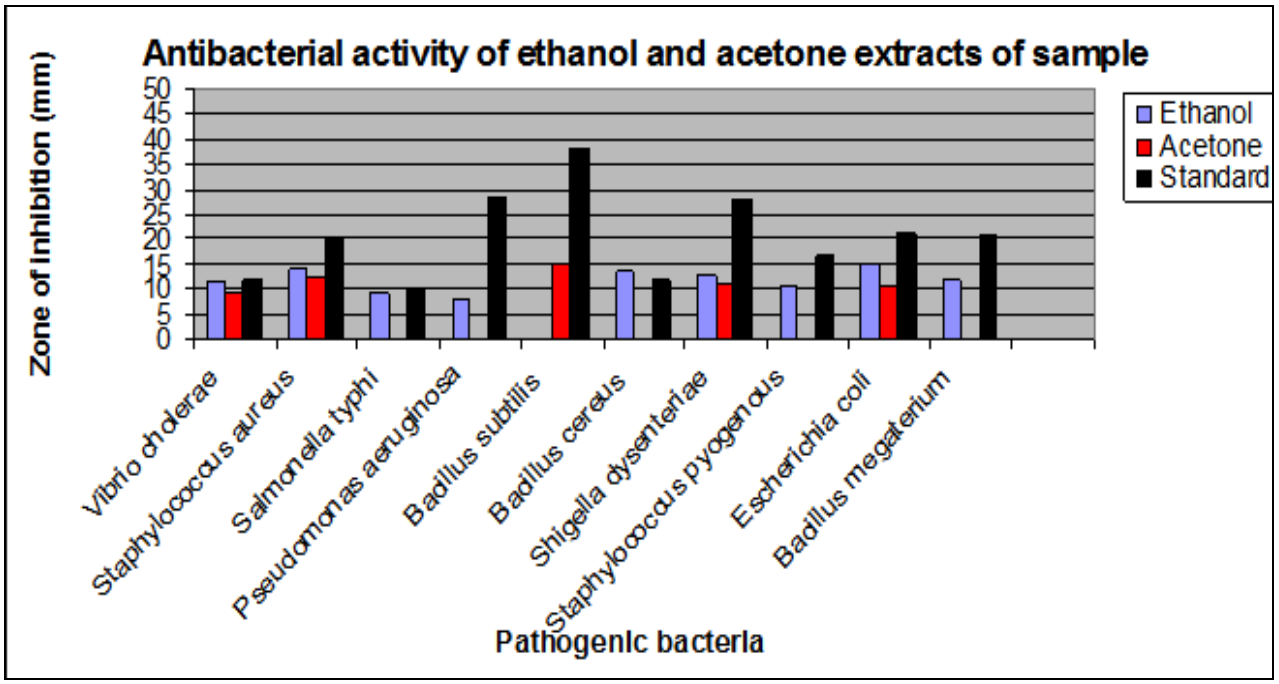

FIGURE 1: ZONE OF INHIBITION OF THE ETHANOL & ACETONE EXTRACTS OF FRUITS OF *TERMINALIA BELERICA*

***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 *Colletotrichum corcori, Fusarium equiseti* and *Candida albicans* organisms. The observed antifungal activity measured in terms of diameter of zone of inhibition in mm (**showed in table 2**).

TABLE 2: *IN VITRO* ANTIFUNGAL ACTIVITY TEST

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
Botroyodiplolia theobromae	NS	NS	11.0
Colletotrichum corcori	11.0	12.0	12.5
Curvularia lunata	NS	NS	NS
Fusarium equiseti	10.5	10.0	13.0
Fusarium solanii	NS	NS	14.5
Aspergillus niger	NS	NS	12.0
Candida albicans	12.0	11.5	13.5
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 *Colletotrichum corcori, 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.

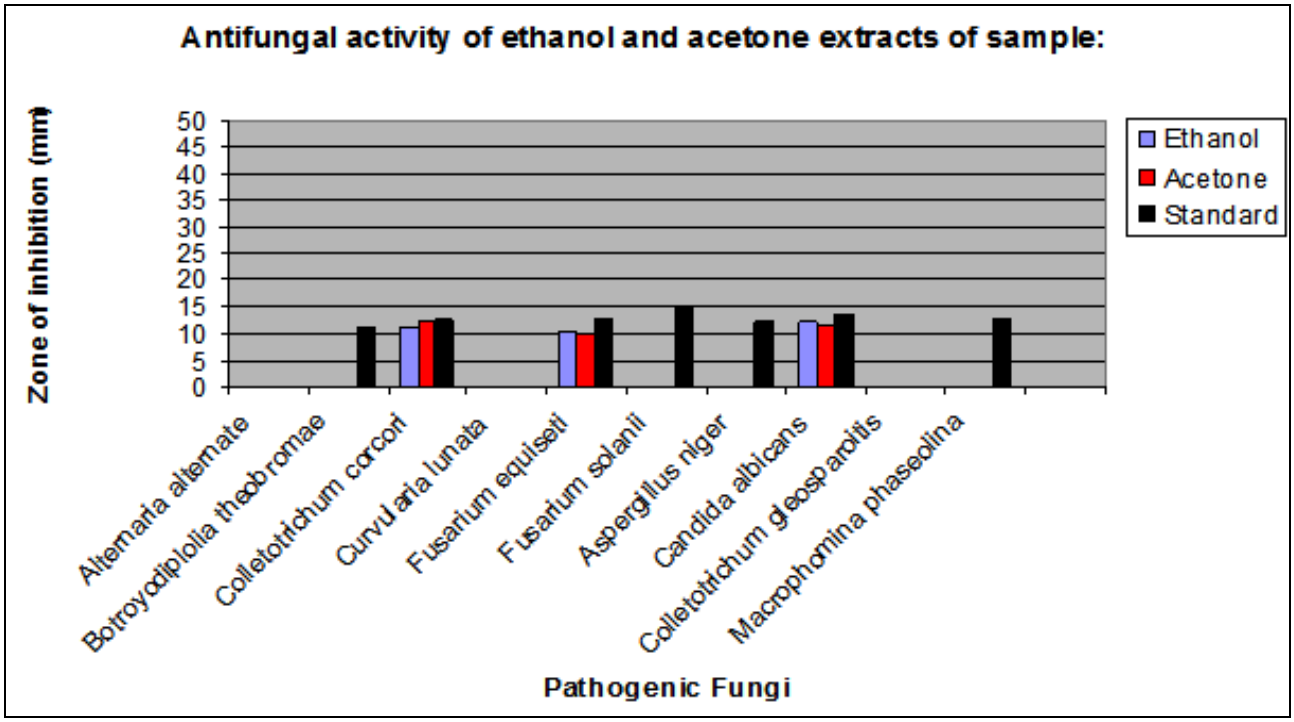

FIGURE 2: ZONE OF INHIBITION OF THE ETHANOL & ACETONE EXTRACTS OF FRUITS OF *EMBLICA OFFICINALIS*

CONCLUSION: *In vitro* antibacterial study of two different solvent extracts of fruits of *Terminalia belerica* was done by simple agar diffusion method. In this experiment ten pathogenic bacteria were used. The ethanol extracts exhibited antibacterial activity against nine of the pathogenic organisms and acetone extracts showed activity against five 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 three 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 *T. belerica* has narrow spectrum antibacterial and antifungal property against some pathogenic organism strains in this research work. As evident from the above discussion, fruits of *T. belerica* 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 *T. belerica* should be thoroughly investigated phytochemically to fully exploit its medicinal and pharmaceutical potentialities.

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How to cite this article:
Hossain MM, Hossen SMM, Islam MS, Tanmy TT, Barua A and Alam J: *In vitro* evaluation of Antibacterial and Antifungal properties of *Terminalia belerica*. *Int J Pharm Sci Res*. 2013; 4(1); 260-264.