

**EVALUATION OF THE ANTIMICROBIAL POTENTIAL OF *EMBLICA OFFICINALIS*
AGAINST CLINICAL ISOLATES****Supriya Kore*, Kartik Sachdeva, Hemanshu Katpal, Neelam Shelke and Madhukar Khetmalas**

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*(Corresponding author: E-mail: supriya.kore@dpu.edu.in)**ABSTRACT**

In the present study we want to evaluate the antibacterial, antifungal activity and the qualitative phytochemicals analysis of various solvent extracts of *Embllica officinalis* fruits. Dried powdered fruit pulp was extracted with seven different solvent according to the polarity. Antimicrobial and antifungal activity was investigated on human pathogen isolated from patients. Antimicrobial activity of *Embllica officinali* fruits extracts were tested against Gram positive and Gram negative bacterial strains by observing the zone of inhibition. The bacteria used in the study were *Staphylococcus aureus*, *Salmonella typhii*, *Acinetobacter baumannii*, *Serratia marcescens*, *Shigella flexneri*, *Escherichia coli*, *Shigella dysenteriae*, *Pseudomonas aeruginosa* and *Candida albicans*. It was observed that acetone, ethanol and methanol extracts showed maximum inhibitory effect on all tested bacteria compared to other solvent extract. The acetone extracts were active against all Gram positive, Gram negative and fungal culture. The extracts exhibited activities with zones of inhibition ranging from 7 mm to 25 mm. Results of the phytochemical studies revealed the presence of reducing sugars, Saponins and alkaloids. We conclude that *Embllica officinalis* seed extract has an active against human pathogens.

KEYWORDS: Antimicrobial activity, *Embllica officinalis*, phytochemical, Zone of Inhibition and Pathogens**INTRODUCTION**

Human infections particularly those involving bacteria, fungi, viruses, nematodes cause serious damages in tropical and subtropical countries of the world. From the last centuries, intensive efforts have been made to discover clinically useful antimicrobial drugs (Ahmed *et al.*, 1998). World health Organization estimated that 80% of the population of developing countries still relies on traditional medicines, mostly plant drugs for their primary health care needs (WHO 2000). Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties. These have been used for strengthen the immune system as well as to cure various ailments. Plants are representing reservoir of effective chemotherapeutic and can provide valuable sources of natural antimicrobial (Balandrin *et al.*, 1985). In recent years antimicrobial/antifungal properties of medicinal plants are being increasingly reported from different parts of the world (Onwukaeme *et al.*, 2007). Natural antimicrobial compounds present in stem, leaves, flower and fruits of plant (Gorden and David, 2001). These antimicrobial substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered as candidates for developing new antimicrobial drugs. *Embllica officinalis* (Amla) has been used traditionally for its tonifying, antiageing and immune enhancing properties (Lalla *et al.*, 2001). It is a flowering plant belonging to Magnoliopsida class. It's of Malpighiales order and belongs to Phyllanthaceae family. It is found all over the India, Pakistan, Bangladesh, China, Sri Lanka and Malayan peninsula. Amla plants leaves, bark, fruit, and root used to cure diseases (Dey, 1980). The fruit is the most commonly used plant part contains up to 700mg vitamin C. The aim of the present study was to evaluate the antimicrobial activity of *Embllica officinalis* against various human pathogens.

MATERIALS AND METHODS

Collection of the plant material *Embllica officinalis* fruits were collected washed with fresh water, fruits were sliced and the seed separated. Sliced fruit parts were dried under shade at room temperature and grinded to powder using and electronic blender.

Preparation of solvent extracts: Based on the polarity of the solvents were selected for extraction of the plant material. Solvent used Hexane, Toluene, Chloroform, Acetone, Ethanol, Methanol and Water. Extraction was done at room temperature. Ten grams of dried ground plant materials were soaked in 100 ml of each of the solvent. After 24h

the plant material were passed through Watman's filter paper No 1. Filtrates obtained were concentrated under vacuum on rotary evaporator.

Antimicrobial assay

Collection of Clinical Isolates: Clinical isolates *Staphylococcus aureus*, *Salmonella typhi*, *Acinetobacter baumannii*, *Serratia marcescens*, *Shigella flexneri*, *Escherichia coli*, *Shigella dysenteriae*, *Pseudomonas aeruginosa* and *Candida albicans* were collected from Dr. D.Y. Patil Medical College, Pimpri, Pune. Most of the strains were isolated from patients with skin infections, urine and some from blood samples.

Inoculum Preparation: Four or five colonies from pure culture of each test organism were transformed to 5ml of Muller Hinton Broth. Broth was incubated at 35-37°C for 18- 24 hours. Colony forming unit of culture 10⁶ CFU/ml

Agar disc diffusion assay: A disc diffusion assay was performed to determine antimicrobial activity (NCCL 2002). Muller Hinton agar and Sabouraud dextrose agar was inoculated with 200µl of microbial cell suspension and pour on sterile Petri dishes. Sterile Whatman's filter No 1 paper discs of diameter 6mm were impregnated with 20µl of extract. Standard antibiotics Streptomycin, Chloramphenicol and Nystatin (10µg/disc) used as positive control and negative control as pure solvent. Plates were incubated overnight at 37°C for 24 hours. In contrast *C. albicans* was incubated at 28-30°C for 48 hours. Resulting zone of inhibition was measured in mm. Each experiment tested in triplicate.

Statistical analysis

Mean zone of inhibition and standard deviation were calculated.

Phytochemical Test for *Emblia officinalis* extracts (Joshi *et al.*, 2011)

Phytochemical tests were performed to detect the presence of major phytoconstituents in the plant extract.

Test for Reducing Sugars: Fehling's test was performed by adding diluted extract drop wise to 5 ml of preheated working Fehling's solution. Color change to brick red is indicative of reducing sugars.

Test for Saponins: Frothing test was performed by adding 20ml water to 1 ml concentrated extract and shaking for 15 min in a test tube. Presence of thick persistent froth was checked, which indicates positive results.

Test for Anthraquinone Derivatives: Borntrager's test was performed by adding 1 ml of 10% ammonia to 2ml extract was added and then mixer was shaken. Change to pink in ammoniacal lower layer which indicates the presence of anthraquinone derivatives.

Test for Flavonoids: The plant extracts was treated with dilute NaOH, followed by treating with dilute HCl. The above solution was checked for a color change and solubility. Yellowish solution with NaOH, which turns to colorless with dilute HCl confirms the positive result.

Test for Tannins: All the three extracts were treated with 15 % ferric chloride test solution. The resultant colour was noted a blue colour indicates the condensed tannins a green colour indicate hydrolysable tannins.

Test for Alkaloids: Two ml of 100 mg/ml solution of extracts stirred with few drops of dilute HCl was added to Mayer's reagent. Creamish color precipitated indicates presence of alkaloids.

Test for phenols: To the test solution few drops of 2% ferric chloride solution were added. Blue green or black colouration indicated the presence of phenols

Test for Amino Acids: Amino acid when boiled with few drops of 5% solution of Ninhydrine violet colour turns red upon gentle heating.

Test for proteins: To the test solution add 4% NaOH and few drops of 1% CuSo₄ solution. Violet colour indicates the presence of proteins.

Test for Steroids: Add three drops of unhydride and 1 drop of concentrated sulphuric acid to 1ml from each of the test solution. Change in colour from deep green to brown to dark brown is an indication of the presence of steroids (Clarke 1975).

Thin Layer Chromatography (Ahmad and Beg 2001)

The acetone ethanol and methanol extracted compound was dissolved in appropriate solvent. 5µl reference solution of Ascorbic acid was applied to silica gel G plates 20X20cm, 0.25mm thickness. Plates were developed using the solvent system Acetone: Alcohol (1:1) and separated zones were visualized using UV light. *R_f* for each spot was measured.

RESULTS AND DISCUSSION

In present study seven different solvent extracts of *Embllica officinalis* were tested against eight human pathogens. Out of the seven solvent all solvent extracts of *Embllica officinalis* show antimicrobial activity by inhibiting one or more number of bacterial isolate. Hexane and toluene inhibited only one organism out of the nine microbial species *Salmonella typhii* and *Shigella flexneri* respectively. Water extracts show antibacterial activity to Gram positive and Gram negative bacteria *Staphylococcus aureus* and *Acinetobacter baumannii*. Screening of the solvent extracts for antimicrobial activity encouraging as methanol, ethanol and acetone extracts show inhibition of maximum number of isolates. Compare to hexane and toluene chloroform show inhibition to two different bacterial samples. Methanol extract inhibited four different isolates and ethanol inhibited six different isolates. Nair and Chanda (2007) reported that *Embllica officinalis* compared to aqueous extract active against all the tested bacterial species used the studies. Acetone extract of the fruit showed inhibition to all the microbial pathogen except *Shigella flexneri*. Acetone found to be the best solvent for extraction of the antimicrobial compounds from the plant fruits. Figure 1 shows the results of the zone of inhibitions to different solvent extract of *Embllica officinalis* to *S. aureus*.

Out of seven five extracts are active against *Salmonella typhii*. Maximum size of inhibition was observed to ethanol extract to *Salmonella typhii*. *Embllica officinalis* acetone extract inhibits fungal species *Candida albicans*. Results of the antimicrobial activity of the *Embllica officinalis* were presented in Table 1. The results of the standard antibiotics Streptomycin, Chloramphenicol and Nystatin also calculated and represented in the Table 1 and Fig 1. Saeed and Tariq (2007) found that *E. officinalis* active against Gram negative bacteria and *C. albicans*. Similarly study by Satish et al., (2008) reported that *E. officinalis* aqueous extract against few human pathogenic bacteria. Results of the qualitative phytochemical analysis of methanol ethanol and acetone extract of *Embllica officinalis* demonstrate the presence of common phytoconstituent like phenol, flavonoid, glycosides, tannins, saponins and proteins. Results of the phytochemical were represented in Table 2. Aqueous extract of the fruits for the presence of the phytochemicals were studied by Meena *et al.*, (2010). The results for phytochemicals may be depending on the collection time and the extraction procedure. As this is the first report on the polar and non-polar solvent extracts

Table 1. Antimicrobial activity of the *Embllica officinalis* fruit extract with seven different solvents.

Solvent Extract	Mean zone of inhibition ± standard deviation								
	<i>S. typhii</i>	<i>S aureus</i>	<i>E coli</i>	<i>Acinetobacter baumannii</i>	<i>Shigella flexneri</i>	<i>Shigella dysenteriae</i>	<i>Pseudomonas sp</i>	<i>Serratia marcescens</i>	<i>Candida albicans</i>
Water	NI	16±2.6	NI	6.66±0.5	NI	NI	NI	NI	NI
Methanol	21 ±2.6	NI	9.3±0.5	NI	14.33±2.08	NI	NI	NI	NI
Ethanol	25.3±1.5	14±1	9.3±0.5	15.33±1.5	10 ±1	17.33±0.57	NI	NI	NI
Acetone	11.33±0.5	23±1.5	10.6±1.15	16.33±1.5	NI	23.66±0.57	12.66±0.5	13±1	12.66±0.5
Chloroform	14± 1	NI	NI	NI	NI	7±1	NI	NI	NI
Toluene	NI	NI	NI	NI	19±1	NI	NI	NI	NI
Hexane	13±1	NI	NI	NI	NI	NI	NI	NI	NI
Streptomycin	17±00	21±1	18±1	20	15.33±1.5	12.66±0.57	15.66±2.08	10.6±1.15	NI
Chloramphenicol	NI	13.66±0.70	12±00	00	7±0.70	13±0.70	11.66±0.70	10.66±00	NI
Nystatin	NT	NT	NT	NT	NT	NT	NT	NT	18±2.081
-ve controls	NI	NI	NI	NI	NI	NI	NI	NI	NI

NI=No Inhibition, NT= Not Tested – Control= respective solvent

Table 2. Phytochemical analysis of *Emblica officinalis* fruit extracts.

Phytochemicals	Methanolic extract	Alcoholic extract	Acetone extract
Reducing Sugars	-	+	+
Saponin	+	+	-
Anthroquinones Derevatives	-	-	+
Flavonoids	-	+	+
Tannins	+	+	-
Alkaloids	+	-	+
Amino Acids	+	+	+
Proteins	+	+	+
Phenols	+	-	+
Steroid	+	+	+

+ Present –Absent

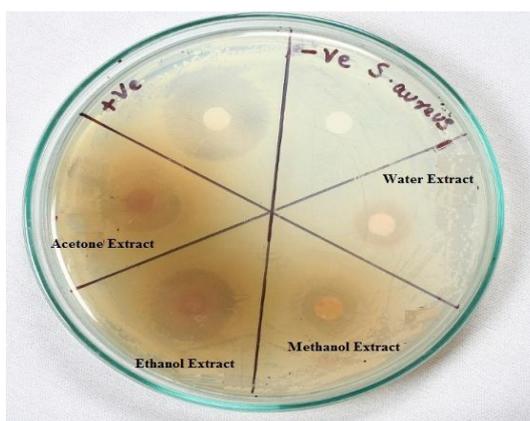


Figure 1. Antimicrobial activity of *Emblica officinalis* against *Staphylococcus aureus*

to be studied for the antimicrobials and phytochemical study. These phytochemical present in the fruit extract may be responsible for the antimicrobial activity. Thin layer chromatography of the three different extract and standard showed three major substances. The R_f value of Methanol extract was 0.5, ethanol 0.7 and acetone extract 0.6 compared to standard to ascorbic acid Present study reveals the importance of natural products to control pathogenic bacteria which can be a threat to human health and can serve to developing inexpensive safe and effective medicine.

ACKNOWLEDGEMENT

Authors are grateful to Dr. D. Y. Patil Vidyapeeth for the financial assistance.

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