

# Antimicrobial activity of *Emblica officinalis*, *Saraca indica* and *Terminalia arjuna* against Multi-Drug Resistant (MDR) Bacterial Pathogens

## Çoklu-İlaç Dirençli Bakteriyal Patojenlere Karşı *Emblica officinalis*, *Saraca indica* ve *Terminalia arjuna*'nın Antimikrobiyal Aktivitesi

Short Communication

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### ABSTRACT

Plant based antimicrobials represent a vast untapped source of medicines and has enormous therapeutic potential. In the present investigation three commonly available plants were screened namely *Emblica officinalis*, *Saraca indica* and *Terminalia arjuna* for antimicrobial activity against four Multi-Drug Resistant (MDR) strains, namely *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus aureus*. Antimicrobial activity of aqueous and ethanol extracts was determined with the help of agar well diffusion method. Result showed that the ethanolic extract appears to have more antimicrobial activity in comparison to the aqueous extract and leaf extract of *E.officinalis* showed maximum antimicrobial activity in comparison to the leaf extracts of *S.indica* and *T.arjuna*. This study highlights that crude extracts of *E. officinalis*, *S. indica* and *T. arjuna* are effective against MDR pathogens. Further study on purification of active ingredient and their efficacy in controlling MDR is in progress.

### Key Words

*Emblica officinalis*, *Saraca indica*, *Terminalia arjuna*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus* and antimicrobial activity

### ÖZET

Bitki temelli antimikrobiyal kullanılmayan muazzam bir ilaç kaynağını temsil eder ve ciddi anlamda tedavi potansiyali vardır. Bu çalışmada, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* ve *Staphylococcus aureus* olarak adlandırılan Çoklu-İlaç'a Dirençli dört yapıya karşı *Emblica officinalis*, *Saraca indica* ve *Terminalia arjuna* antimikrobiyal aktivitesi gözlemlendi. Sulu çözelti ve etanol özütlerinin antimikrobiyal aktivitesi katı difüzyon yöntemiyle belirlendi. Sonuçlar, etanol özütlerin sulu çözeltilere göre ve *E.officinalis* yaprak özütlerinin *S.indica* ve *T.arjuna* yaprak özütlerine göre daha çok antimikrobiyal aktivite gösterdiklerini ortaya koymuştur. Bu çalışmada, işlenmemiş *E. officinalis*, *S. indica* ve *T. arjuna* özütlerinin MDR patojenlerine karşı etkisi vurgulanmaktadır. Aktif bileşenlerin saflaştırılması ve MDR kontrolündeki etkileri ile ilgili başka çalışmalar devam etmektedir.

### Anahtar Kelimeler

*Emblica officinalis*, *Saraca indica*, *Terminalia arjuna*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus* ve antimikrobiyal aktivite.

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## INTRODUCTION

The revival of interest in herbal drugs is mainly due to current widespread belief that “green medicine” is safe and dependable than the expensive synthetic drugs, many of which have adverse effects [1]. In recent years, research on medicinal plants has attracted a lot of attentions globally. Large body of evidence has accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary and alternate systems of treatment of human diseases. Plants are rich in wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavanoids etc, which are found to have antimicrobial properties [2]. The secondary products may exert their action by endogenous metabolites, ligands, hormones, signal transduction molecules or neurotransmitters and thus have beneficial effects on humans due to similarities in their potential target sites [3].

To the emerging problem of antibiotic resistance, phytochemicals obtained from medicinal plants may be one of the remedy. This further drives the need to screen medicinal plants for novel bioactive compounds as plant based drugs are biodegradable, safe and fewer side effects [4].

Hence this study is undertaken to screen the antimicrobial activity of three commonly available plants namely *Emblica officinalis*, *Saraca indica* and *Terminalia arjuna* against four Multi-Drug Resistant (MDR) pathogens, namely *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus aureus*.

## MATERIALS AND METHODS

For the present study the leaves of the three plants were collected, dried and crushed to fine powder. The aqueous and ethanolic extract was prepared. Aqueous extract was prepared by soaking leaf powder in 50 ml of sterile distilled water and shaken at 120 rpm at room temperature for 4 hours. The mixture was then steam-sterilized for 5 min and extract was collected by squeezing through a sterile muslin cloth. The aqueous extract was used immediately. The ethanolic extract was prepared by adding 5 gram of powdered material to 50 ml of ethanol (70% v/v) and soaked for 24 hours. The mixture was then

filtered by a muslin cloth and kept for evaporation at room temperature. The evaporated filtrate was then diluted DMSO, i.e. Dimethyl Sulphoxide.

MDR bacterial cultures were obtained from “Microbial Culture Collection Bank”, Department of Microbiology and Microbial Technology, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad and were maintained on nutrient agar slants and stored at 4°C. The Multidrug resistance pattern of bacterial cultures is shown in Table 1.

Sterilized nutrient broth was inoculated with cultures and inoculated tubes were incubated at 37°C for 24 hours. Overnight broth cultures of the test organism were swabbed uniformly on the surface of the nutrient agar media using sterile cotton swabs. Four wells were cut out using a sterile stainless steel cork borer and wells were filled with 0.1 ml of leaf extract. For aqueous extract distilled water and for ethanolic extract DMSO, respectively was used as control. The plates were incubated at 37°C for 24 hours. Zone of inhibition formed around the wells was observed and measured in millimeters.

## RESULTS AND DISCUSSION

In the present investigation it was found that the ethanolic extract appears to have more antimicrobial activity in comparison to the aqueous extract. The antimicrobial activity of the three plants against MDR strains is shown in Table 2. The reason may be due to nature of biologically active components such as alkaloids, saponins, tannins, phenols etc. could be enhanced in presence of ethanol [5].

Aqueous extracts of all the plants studied showed negligible antimicrobial activity against *E.coli* and *P. aeruginosa*. Ethanolic extract of *E.officinalis* showed highly significant ( $p > 0.001$ ) antimicrobial activity against *K. pneumoniae* and *E.coli* than aqueous extract. The antimicrobial activity of *E.officinalis* has been attributed to presence of flavanoids, tannins and glycosides where leaves contain gallic acid, elagic chebulic acid, chebulinic acid, chebulagic acid, a gallantonic acid called amlic acid, alkaloids, phyllantidine and phyllantine [6]. Significant ( $p > 0.01$ ) antimicrobial activity of ethanolic extract of *S.indica* was noted against *P.aeruginosa* and *E.coli* as compared to aqueous extract. The phytoconstituents in leaves

**Table 1.** Multi-drug resistance pattern of bacterial cultures.

S.No.	Bacterial Pathogens	Resistant to Antibiotics
1	<i>Pseudomonas aeruginosa</i> (MCCB0035)	Kanamycin, Nalidixic acid, Chloramphenicol, Aztreonam and Nitrofurantoin.
2	<i>Klebsiella pneumonia</i> (MCCB0019)	Carbenicillin, Ceftriaxome, Tobramycin, Gentamycin and Nitrofurantoin.
3	<i>Escherichia coli</i> (MCCB0124)	Amoxicillin, Carbenicillin, Erythromycin, Penicillin, Tetracycline and Vancomycin.
4	<i>Staphylococcus aureus</i> (MCCB0066)	Penicillin, Erythromycin, Methicillin and Clendamyacin

**Table 2.** Antimicrobial activity of three medicinal plants against MDR strains

Bacterial pathogens	Zone of inhibition (mm)#					
	<i>Emblica officinalis</i> (Amla)		<i>Saraca indica</i> (Ashoka)		<i>Terminalia arjuna</i> (Arjun)	
	Aqueous extract	Ethanollic extract	Aqueous extract	Ethanollic extract	Aqueous extract	Ethanollic extract
<i>Pseudomonas aeruginosa</i>	15	23*	0	20**	0	15*
<i>Klebsiella pneumoniae</i>	12	35***	10	20*	0	15*
<i>Escherichia coli</i>	7	29***	0	25**	0	15*
<i>Staphylococcus aureus</i>	15	33**	17	23*	15	15*

\*:  $p > 0.05$  (less significant); \*\*:  $p > 0.01$  (more significant); \*\*\*:  $p > 0.001$  (most significant)

#: zone of inhibition was measured with the diameter of well.

of Ashoka and found to have glycosides, flavanoids, tannins and saponins [7]. These phytoconstituents are responsible for various therapeutic effects of *S.indica* leaves. Aqueous extract of *T.arjuna* showed activity only against *S.aureus* but significant difference ( $p > 0.05$ ) was seen in ethanolic extract against *P.aeruginosa*, *K.pneumoniae* and *E.coli*. Leaves of *T.arjuna* contain flavanoids called luteolin [8]. Leaf extract of *E.officinalis* showed significant antimicrobial activity followed by *S.indica*. However *T.arjuna* showed least antimicrobial activity among the plant studied. From the results of this investigation it is concluded that crude extracts of *E. officinalis*, *S. indica* and *T. arjuna* are effective against MDR bacterial pathogens.

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