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Research Article

COMPARING THE ANTIMICROBIAL ACTIVITIES OF EMBLICA OFFICINALIS, TERMINALIA CHEBULA AND TERMINALIA BELLERICA AGAINST PATHOGENIC BACTERIA

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A B S T R A C T

Antimicrobial resistance has been deemed as one of the biggest upcoming catastrophes. Yet, there are rarely new antibiotics available to treat new and resistant infections. This creates a void that can be filled by alternatives to antibiotics developed from cheaper and more readily available sources. Plants have been a source of medication and plant extracts have shown potential to serve as alternatives to or sources of antibiotics and antimicrobials. Medicinal fruits such as Amalaki (*Emblica officinalis*), Bahera (*Terminalia bellerica*) and Haritaki (*Terminalia chebula*) have long been used in combination or separately to treat various diseases. Thus, the alcohol extracts of these fruits were tested against eight pathogenic bacteria *Staphylococcus aureus, EAEC, ETEC, Enterobacter cloacae, Shigella flexneri, Enterococcus faecalis, Klebsiella* and *Pseudomonas aeruginosa.* The procedure involved extraction with Soxhlet apparatus followed by testing the antimicrobial potential of the obtained crude extract against the bacteria using agar well diffusion method. All the extracts produced significant activity against all the bacteria. The highest zone of inhibition was found in *E.officinalis* ethanol extract on EAEC (31.67mm). The highest activity index was found for *T.chebula* ethanol extract on S.flexneri(2.20). The results depict a possibility of developing antimicrobial drugs from these medicinal plants.

INTRODUCTION

The first global report on antibiotic resistance by WHO [17] increased the tension regarding antimicrobial resistance by stating that death by antimicrobial resistance will increase to ten million in 2050. With the growing problem of microbial resistance and a lack of antibiotics to address the ever changing status of infections, it becomes a matter of utmost priority to find a different path to combat antimicrobial resistance.

According to a report by WHO [16], 80% of the world population relied on natural sources for effective medication. Plant medicines are particularly popular in developing countries due to their cost effectiveness as well [1]. Three of the most frequently used and effective medicinal plants in Bangladesh are *Emblica officinalis (*or *Phyllanthus emblica), Terminalia chebula* and *Terminalia bellerica.*

The fruits of *E. officinalis* contain a higher amount of vitamin C [14], protein, and amino acids like glutamic acid, hydrolysable tannins, alkaloids, gallic acid, ellagic acid, chebulinic acid, and chebulagic acid [15, 5]. *T.chebula* is particularly rich in different kinds of pyrogallol type hydrolysable tannins like aregallic acid, chebulic acid, punicalagin, chebulanin among many more [2]. In fact, *T.chebula* contains more phenolics than any other plant [8]. *T.bellerica* has a number of phytoconstituents which may contribute to its therapeutic properties. It contains bellericanin which is a glucoside. It also contains Gallo-tannic acid, Ellargic Acid and gallic acid lignans [3, 6, 10].

Since these plants contain a range of different phytoconstituents, research can be conducted to provide a solution to the problem of antibiotic resistance using these plants. Thus alcohol extracts of the fruits of *E.officinalis, T.chebula* and *T. bellerica* were tested for their antimicrobial potential on eight pathogenic bacteria.

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MATERIALS AND METHODS

Collection and Processing

Fresh fruits of *E. officinalis, T.chebula,* and *T. bellerica* were deseeded, cut into fine thin pieces, dried to a crisp in sunlight, and ground to a fine powder.

Extraction

For extraction, 75 grams of powdered pulp and 250 ml of ethanol was undergone Soxhlet extraction at 72°C which was continued until the cotton turned colourless. The solvent was removed from the mixture using a rotary evaporator at 80°C. This process was repeated for each fruit using methanol as a solvent and the Soxhlet apparatus was run at 60°C in this case.

Collection of bacterial strains

The following strains of bacteria were used: *Staphylococcus aureus, Enteroaggregative E. coli (EAEC), Enterotoxigenic E. coli (ETEC), Enterobacter cloacae, Shigella flexineri, Enterococcus faecalis, Klebsiella,* and *Pseudomonas aeruginosa.* All strains of bacteria were maintained in laboratory fridge through regular subcultures.

Agar well diffusion

From each bacterial suspension, using a sterile cotton swab, a lawn culture was done on MHA media. Each well was then filled with 30 microliters of ethanol extract, methanol extract and distilled water respectively. Antibiotic Norfloxacin was used for positive control. The plates were incubated for 24 hours at 37°C. After 24 hours, the zones of inhibition were measured and recorded. From this data, activity index of the extracts were calculated using the formula:

$$\textit{Activity Index (AI)} = \frac{\textit{Zone of inhibition of fruit extract(mm)}}{\textit{Zone of inhibition of Antibiotic(mm)}}$$

RESULTS AND DISCUSSION

The largest zone of inhibition was observed on EAEC by the ethanol extracts of *E. offcinalis* (31.67 mm). However, in comparison of the three fruits, *T.chebula* produced the most consistent results. It produced the largest zones of inhibition in *P.aeruginosa, Klebsiella species, E.faecalis, S.flexneri* and ETEC (23.67mm, 20.00mm, 27.33mm, 23.67mm and 30 mm) by both of its extracts. In case of *E. cloacae,* the methanol extract of the fruit exhibited the highest zone of inhibition (21 mm). Generally, ethanol extracts performed better than methanol extracts and in the case of *T.chebula* and *E.officinalis,* the results of the methanol extracts were only slightly lesser than that of the ethanol extracts.

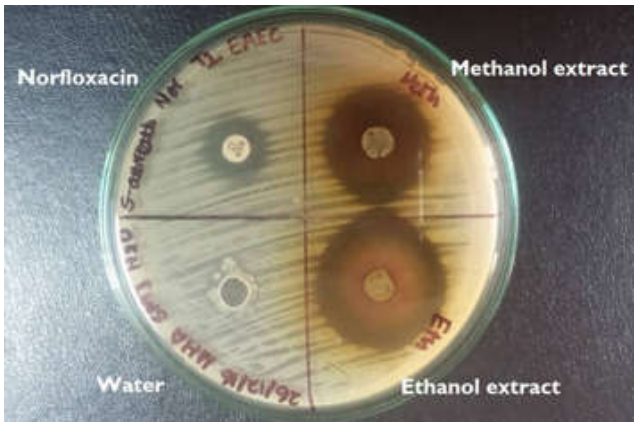

Figure 1 Effect of *E.officinalis* extracts on *EAEC*

Comparison of the activity index showed that the ethanol and methanol extracts of *T. chebula* produced the highest activity. In fact, both types of extracts of the fruits were more than twice as effective as Norfloxacin. Although slightly less than these two extracts, the ethanol extract of *E. offcinalis* produced a significant amount of activity. All the extracts were more effective than Norfloxacin in the case of *E.faecalis* and *S. flexneri.* The extracts of *E. officinalis* were more effective than Norfloxacin in the case of *EAEC, S.flexneri,* and *E.faecalis.* In the case of *S. aureus,* the ethanol extract was better than the antibiotic and the methanol extract was as effective as Norfloxacin.

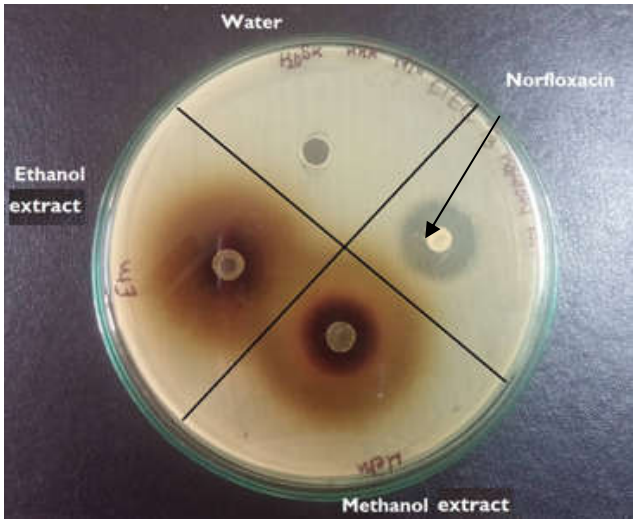

Figure 2 Effect of *T.chebula* extracts on *ETEC*

T.chebula extracts were more effective fo*r E.faecalis, Klebsiella* and significantly for *S. flexneri. T. bellerica* extracts were more effective only in the case *of E. faecalis* and the methanol extract of the fruit performed slightly better than Norfloxacin in the case of *S. flexneri.* Overall, the ethanol extracts exhibited better activity.

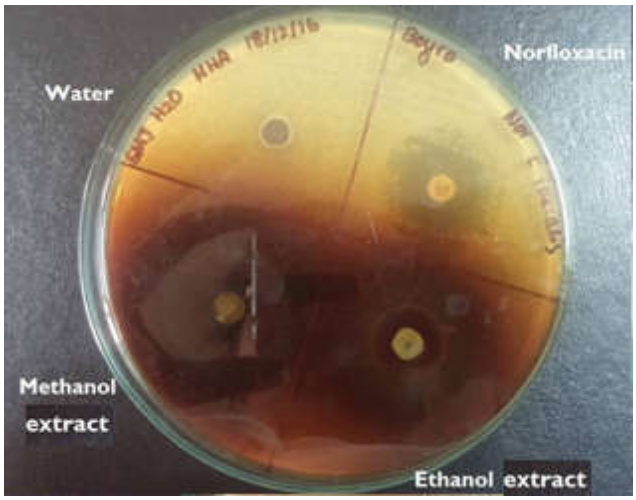

Figure 3 Effect of *T.bellerica* on *E. faecalis*

Although, a lot of studies have been conducted to observe the effects of *E.officinalis, T.chebula,* and *T.bellerica* on *E.coli*, significant and relevant studies were not found that exhibit the effect of the extracts of these fruits on the different pathotypes of *E.coli.* Of the available studies, two studies produced significant antimicrobial activity in both ethanol and methanol extracts of *E.officinalis, T.chebula* and *T. bellerica* [7, 13]. However, the zones of inhibition of this research, exhibited by the extracts of *E.officinalis and T.chebula* were much larger in both EAEC and ETEC compared to the study of Tambekar *et al*. [13]. Considering this research used the same concentration of extracts, both *T.chebula* and *E.officinalis* extracts produced higher zones of inhibition. This trend was also observed in cases of *S.aureus, P.aeruginosa, Klebsiella.* The results were also consistent with the study by Nair *et al*. [4] which observed the effects of the ethanol extract of *E. officinalis* on *P.aeruginosa* and *S.aureus.*

However, the results were different for *T. bellerica*. Compared to the extracts of the other two fruits, *T. bellerica* showed

significantly lower zones of inhibition. This also contradicted the results of the two previously mentioned and another similar study Safiullah *et al*. and Tambekar *et al*. [7, 13]. In a similar research against HIV isolates, which included *Shigella flexneri, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumonia,* it found similar results were found [12]. However, since their alcohol extracts were diluted to 10% some bacteria like *Klebsiella* did not show any results. *Shigella flexneri* and *E. faecalis* produced inhibition zones much higher in this study due to the concentration of the crude extract.

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Table 1 Zones of Inhibition of the alcohol extracts of the medicinal fruits (mm)

Organism	E.officinalis		T.chebula		T. bellerica	
	Ethanol extract	Methanol extract	Ethanol extract	Methanol extract	Ethanol extract	Methanol extract
S.aureus	24.67	22.00	24.67	24.33	16.33	15.00
EAEC	31.67	25.33	20.67	20.67	11.67	10.67
ETEC	19.67	15.00	30.00	25.00	11.67	13.33
E.cloacae	19.67	19.00	18.00	21.00	12.67	11.67
S.flexneri	22.00	18.33	23.67	22.67	14.33	14.67
E.faecalis	23.33	20.00	26.33	27.33	22.67	19.67
Klebsiella spp.	18.00	15.67	20.00	19.00	9.67	10.67
P.aeruginosa	19.00	15.67	23.67	20.67	19.33	19.67

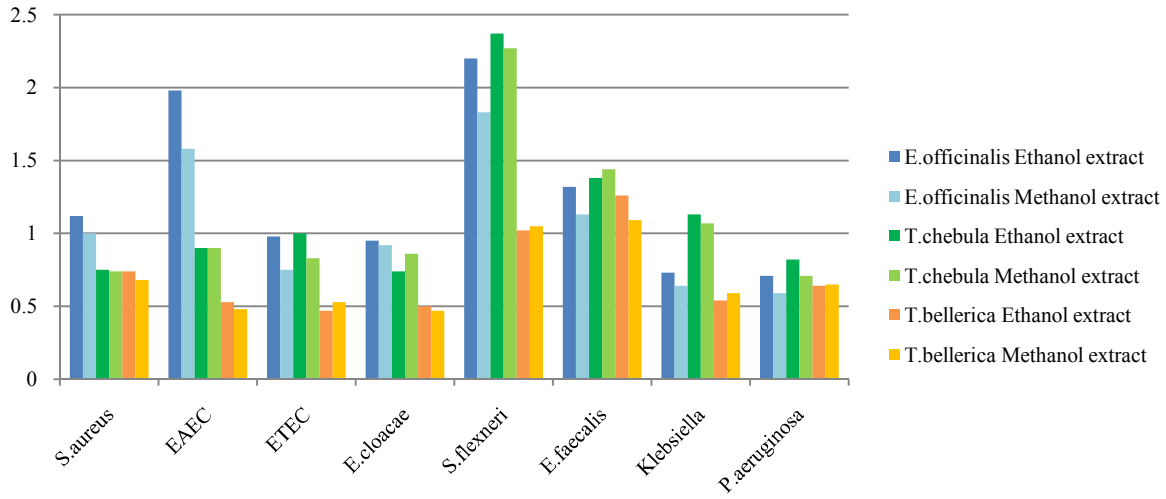

Figure 4 Comparison between the Activity indices of the alcohol extracts of the medicinal fruits on pathogenic bacteria (mm)

There has not been much prior research that can be used as an example to compare the effect of the six extracts on *E.cloacae*. The extensively positive results can be attributed to the presence of phenolic compounds in these fruits. The research with HIV isolates also conducted a phytochemical study, alongside the antimicrobial study. They observed that the extracts of the fruit have a high total phenolic content, flavonoid, and carotenoids [12]. These three fruits have gallic acid as their principal content confirmed through HPLC [10]. Gallic acid and ethyl ester which are effective against methicillin- resistant Staphylococcus have been isolated from ethyl alcohol extracts of *T.chebula* [9]. Since *T.chebula* was extremely effective against *S. aureus* in this research, its effectiveness can be attributed to the presence of gallic acid. Its particularly rich source of phenolics is probably the reason for its better performance than other fruits. A lot of the compounds contained in *T.bellerica* extracts are the same as *E.officinalis* extracts and *T.chebula* extracts.

The outcomes of this research means each of these extracts may prove valuable in further studies, in the development of antibiotics and in the development of substances such as handwash and topical creams that require the use of antimicrobials.

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