Original Article

Effect of Aqueous *Emblica officinalis* (amlaki) Extract on *Staphylococcus aureus*

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Abstract

Objective: The aim of the study was to find out the effect of Aqueous *Emblica officinalis* (amlaki) extract on *Staphylococcus aureus* and to detect the bacterial susceptibility to Aqueous *Emblica officinalis* (amlaki) extract and Gentamicin.

Methods: Bacterial susceptibility of Aqueous *Emblica officinalis* (amlaki) extract on *Staphylococcus aureus* was determined by broth dilution method.

Results: Aqueous amlaki extract has inhibitory effect on growth of *S. aureus* at 15.62 mg/5ml and in subsequent higher concentration. So the MICs value of AAE was 15.62 mg/5ml for *S.aureus*. *S.aureus* was sensitive against gentamicin at 10 µg disc potency with zone Diameter of inhibition more than 15mm. Zone Diameter of inhibition was 17.25±0.27 mm for *S.aureus*.

Conclusions: The data suggest that the plant *Emblica officinalis* (amlaki) has antibacterial effect on *S.aureus* . There is potential possibility of developing this plant as a source of antimicrobial agent.

Keywords: Antibacterial activity, Plant extract, *Emblica officinalis*, Gentamicin.

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Introduction

Herbal Medicines have been known to human for centuries. Practice of traditional medicine have described therapeutics efficacy of many indigenous plants for several disorder. It is popularly known that, herbal medicine have less toxic effect. For this reason, intensive research was carried out over the plant kingdom during the past few decades for helping the economically poor people.[1] Medicinal plants have been found useful in the cure of a number of diseases including bacterial diseases. Medicinal plants are a rich source of antimicrobial agents.[2] Due to a rapid increase in the rate of infections, antibiotic resistance in microorganisms and due to side effects of synthetic antibiotics, medicinal plants are gaining popularity over these drugs.[3] Although medicinal plants produce slow recovery, the therapeutic use of medicinal plant is becoming popular because of their lesser side effects and low resistance in microorganisms.[4] Antimicrobial

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activities of many plants have been reported by the researchers. The antimicrobial activities of medicinal plants can be attributed to the secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids etc. that are present in these plants.[5] The antimicrobial activity of *Emblica officinalis* & *Coriandrum sativum* against Gram positive bacteria & *Candida albicans* was done and the aqueous decoction and infusion of *Emblica officinalis* also exhibited potent antimicrobial activities against all bacterial isolates tested. The aqueous infusion of *Emblica officinalis* showed maximum activity against *B.subtilis* and the aqueous decoction of *Emblica officinalis* showed maximum activity against *S.haemolyticus*. The minimum activities of both aqueous decoction and infusion of *Emblica officinalis* were found against *Candida* albicans.[6] Ten medicinal plants, namely *Commiphora wightii, Hibiscus cannabinus, Anethum gravelens, Emblica officinalis, Ficus religiosa, Ficus racemosa, Ficus benghalensis, Ficus tisela, Mentha arvensis* and *Mimusops elengi,* were screened for potential antibacterial activity against medically important bacterial strains, namely *Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus, Bacillus cereus, Alcaligenes faecalis* and *Salmonella typhimurium. Emblica officinalis* showed strong activity against all the tested bacterial strains.[7]

Materials & Methods

Aseptic precaution was maintained, for sterilization autoclaving and hot air woven was used accordingly.

1. Bacterial isolates and Preparation of microorganism inoculum *Staphylococcus aureus* collected from department of Microbiology, Dhaka Medical College, Dhaka. From 18-24 hour old agar plate one loop full bacteria was taken by a sterile wire loop and mixed in sterile nutrient broth media. Then it was kept in incubator for 18-24 hour at 37 ^{0}C in separate sterile bottle.

2. Plant extract preparation Mature *Emblica officinalis* (amlaki) fruits—bought from local market washed properly with plain water then with distilled water. Then cut into small pieces along with seed then sun dried for 7 days. The extract was prepared in Drug Research Laboratory in Centre for Advance Research in Science (CARS) Dhaka University Dhaka.

3. Broth dilution Ten test tube were taken and numbered serially one gram of extract and 5ml sterile nutrient broth added in the 1st tube mixed thoroughly Then 5 ml from first tube transferred to the 2nd one. This procedure done up to the last test tube [8]. This will give a concentration ranging from 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.9, 1.9 mg/5ml. bacterial inoculum turbidity was matched with Mcfarland standard tube by adding sterile normal saline in the inoculum stock. Then 0.1 ml of inoculum was transferred to each test tube all containing AAE (Aqueous Amlaki Extract) at different concentration. One test tube kept as control which only contains broth and inoculum. The test tubes were placed serially and incubated in incubator at 37^{0}C for overnight. Growth of the organisms were examined.[8]

4. Inoculation from the test-tube in solid media From the test tubes a wire loop full of broth was taken& inoculated in solid media in Petri dish which were marked serially & incubated in incubator at 37^0 C for overnight. After overnight incubation the growth of test organism in solid media were examined**.**

5. Disc diffusion susceptibility The antibiotic discs of Gentamicin contained 10 µg/discs was taken for standardization. The plates were inverted and placed in incubator to 37^{0}C temperature for 18-24 hours.[9, 10]

6. Interpretation of result Minimum inhibitory concentration was recorded from the inoculated test tube. Diameter of zone of inhibition of Gentamicin was recorded.

7. Statistical analysis Test procedure was repeated 6 times then obtained Data. Data was compiled and analyzed by Microsoft excel, Mean and online SD calculator.

Results

AAE (Aqueous Amlaki Extract) had inhibitory effect and *S. aureus*, is susceptible to AAE in nutrient broth and nutrient agar media. AAE has inhibitory effect on growth of *S. aureus* at 15.62 mg/5ml and in subsequent higher concentration. So the MIC value of AAE was 15.62 mg/5ml for S.*aureus* . *S.aureus* was sensitive against gentamicin at 10 µg disc potency with zone Diameter of inhibition more than 15mm. Zone Diameter of inhibition by Gentamicin was 17.25±0.27 mm for *S.aureus*.

Table 1: Effect of AAE on *S. aureus*

No of test tube	Concentration of AEE mg/5ml	***S. aureus***
1	Control No extract	Huge growth
2	500	Growth inhibited
3	250	Growth inhibited
4	125	Growth inhibited
5	62.5	Growth inhibited
6	31.25	Growth inhibited
7	15.62	Growth inhibited*
8	7.81	Growth not inhibited
9	3.9	Growth not inhibited
10	1.9	Growth not inhibited

Table 2: Diameter of inhibition zone (DIZ) of Gentamicin against bacteria

Test bacteria			Gentamicin	
			Disc potency µg/disc	ZDI mm
S. aureus			10	17.25±0.27

ZID - Zone diameter of inhibition

By Gentamicin ZDI was 17.25±0.27 at Disc potency 10 µg/disc.

Discussion

Emblica officinalis which has important pharmacological function have been revised by many researchers. The study was done to determine the effect of aqueous amlaki extract on *S.aureus*. MIC of Aqueous *Emblica officinalis* (Amlaki) extracts was determined by broth macro dilution method (Das et al. 2009).Bacterial susceptibility of Aqueous *Emblica officinalis* (amlaki) extract detected by agar disc diffusion method (AAE was poured in to blank disc) and for standardization, bacterial susceptibility to Gentamicin was done.[9,10]

In this study The Minimum inhibitory concentration of AAE against *S.aureus* was15.62 mg/5ml. The average zone diameter of inhibition by the gentamicin disc was 17.25±0.27 mm for *S. aureus*. Indigenous medicinal plants exhibit antimicrobial properties which stated that *Emblica officinalis* possess antibacterial activity against certain microorganism.[11]

Emblica officinalis Seed and *Nymphaeodorata* stamen extracts, alone and in combination, and in combination with amoxicillin have synergistic anti Staphylococcal activity.[12] Antimicrobial properties and phytochemical analysis of *Emblica officinalis* fruit and leaf extract report stated that aqueous decoction and infusion of crude

powder of *Emblica officinalis* has strongest activity against *S.aureus* than *S.pyogenes* and *K.pneumoniae*. The fruit extract was more effective than the leaf extract in terms of the antimicrobial action of the phytochemicals of their respective extract.[13]

The antimicrobial activity of *Emblica officinalis* & *Coriandrum sativum* against Gram positive bacteria & *Candida albicans* was done and the aqueous decoction and infusion of *Emblica officinalis* also exhibited potent antimicrobial activities against all bacterial isolates tested.[6]

Investigated on antibacterial potential of three medicinal fruits used in triphala (an ayurvedic formulation) and focused on the antibacterial potential of dimethylesulphoxide (DMSO) extracts from fruit of *Emblica officinalis, Tterminalia bellerica* and *Terminalia chebula* against *Salmonella typhi* (32 strains) in agar dilution technique. DMSO extracts showed significant antibacterial activity against 32 strains of *S.typhi.*[14]

Studied on five medicinal plants, namely *Hisbiscus cannabinus, Emblica officinalis, Ficus religiosa, F. racemosa* and *Mimus opselengi* to find potential antibacterial activity against medically important bacterial strains, namely *Pseudomonas aeruginosa, Staphylococcus aereus, Bacillus cereus, E. coli* and *Salmonella typhimurium*. The Antibacterial activity was determined in aqueous and ethanol extracts using agar well diffusion method. *E. officinalis* showed stronger activity than the other plants against.[15]

In-vitro study of anti-*Helicobacter pylori* activity of *Emblica officinalis* where researcher studied the phytochemical (phenolic, flavonoids and carotenoid) content and anti-*H.pylori* activity of various extracts (petroleum ether, ethanol, and aqueous) of *Emblica officinalis*. This study revealed that the ethanolic and aqueous extracts of *E.offficinalis* show anti-*H.pylori* activity.[16]

Antibacterial activity of aqueous herbal extracts of *Emblica officinalis* against a total of 20 clinical *Klebshiella sp*. isolates and found *Emblica officinalis* exhibits antibacterial properties against *Klebshiella sp*. Isolates.[17]

The tannins isolated from leaves and fruits of *E. officinalis* showed antibacterial activity against *E. coli, P. aeruginosa, B. subtilis, S. boydii, S. flexneri, S. aureus* and *S. epidermidis*. The tannins isolated from leaves exhibited maximum antibacterial activity than fruits tannins.[18]

Study of in vitro antibacterial activity of *E. officinalis* against *E. coli, Klebsiella, S.aureus, P. multocida* and found that *E. officinalis* has inhibitory effect.[19]

The findings suggest that the plant *Emblica officinalis* (amlaki) has antibacterial effect on *S.aureus* . There is potential possibility of developing this plant as a source of antimicrobial agent. *S.aureus* was sensitive against Gentamicin. Medicinal plants are being used for centuries and they have shown effectiveness in alleviating diseases and herbal plants are natural and has less side effect. So more scientific study on *Emblica officinalis* (amlaki) plant in order to determine therapeutic application, mechanism of action, therapeutic dose, toxicity profile and other related aspects is needed.

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Conflict of Interest

We declare that we have no conflict of interest.

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