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Antibacterial effect of Amlaki (*Phyllanthus emblica*) extract against *Pseudomonas aeruginosa*

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Keywords: Antibacterial effect; *Phyllanthus emblica* (Amlaki); *Pseudomonas aeruginosa*; Aqueous and ethanolic extract.

Abstract

Background: The emergence of bacterial resistance is creating a global health issue. The newer generations of antimicrobials used to combat this problem are expensive and their adverse effects are also notable. Natural herbal remedies have shown promising antimicrobial properties and fewer side effects compared to synthetic antimicrobial agents. In this regard, one of the reputed medicinal plants, *Phyllanthus emblica* (Amlaki) was investigated for potential antibacterial effect against the most common nosocomial pathogen (*Pseudomonas aeruginosa).*

Objective: The experimental study was to determine the antibacterial effect of aqueous as well as ethanolic extract of (*P. aeruginosa*) against standard strains of *P. aeruginosa.*

Methods: The study was conducted during the period of July 2018 to June 2019 in the Department of Pharmacology and Therapeutics in collaboration of Department of Microbiology, Mymensingh Medical College, Mymensingh, Bangladesh. Six separate experiments were done e.g. (Expt-I) Determination of the antibacterial activity of aqueous extract of Amlaki (AAE) and (Expt-II) ethanolic extract (EAE) against P. aeruginosa by Kirby-Bauer disc diffusion method, (Expt-III) Determination of minimum inhibitory concentration (MIC) of aqueous extract, (Expt-IV) ethanolic extract and (Expt-V) a standard antibiotic ciprofloxacin against test organism by broth dilution technique as well as making a comparison with MIC of AAE and EAE and (Expt-VI) Subculture studies of materials from effective AAE, EAE and ciprofloxacin preparations for confirmation of respective results of Experiments III, IV and V.

Result: Both aqueous and ethanolic extract of Amlaki was found active against *P. aeruginosa* in disc diffusion method. Aqueous and ethanolic extracts were used in six different concentrations 100 µg/ml, 200 µg/ml, 400 µg/ml, 600 µg/ml, 800 µg/ml and 1000 µg/ml. For aqueous extract and ethanolic extracts dose dependent inhibitory effect was observed against the test organism. Showing greater inhibitory effect at the same concentrations [AAE vs EAE: 6vs6, 6vs8, 7.5vs17, 15.5vs23.5, 24vs25, 27.5vs35]. The MIC of ciprofloxacin was lowest in comparison to MICs of AAE and EAE for the test organism. The subculture studies also confirmed the results of the previous experiments.

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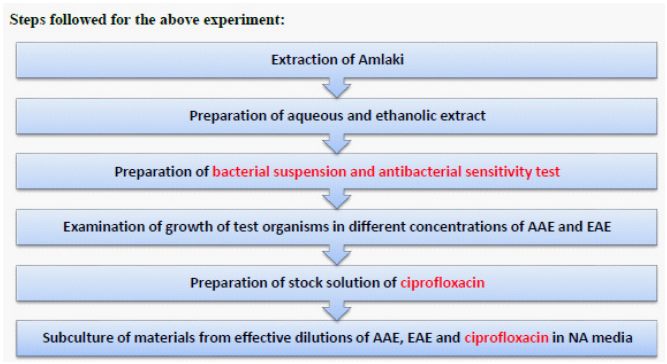
Conclusion: This study demonstrates that there is definite antibacterial effect of both aqueous and ethanolic extract of *P. emblica* (Amlaki) against *P. aeruginosa*. Detection and isolation of the biologically active ingredients responsible for this antibacterial effect demands further large scale study.

Introduction

Infectious diseases are the leading cause of untimely death world-wide and it has become a global concern. The clinical efficacy of many existing antibiotics is being threatened by rapid emergence of multidrug-resistant pathogens. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases [1]. Different species of medicinal plants have been analyzed for biologically active ingredients known to have pharmacological properties and many of the studied plants have shown antimicrobial property [2]. Plant extracts highlight a continuous effort aimed at finding new compounds against microorganisms. The use of plant extracts, with known antimicrobial properties, can be of great significance in the treatment of various microbial infection [3]. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects [4]. Therefore, much attention has been paid to the discovery and development of new antimicrobial agents of plant origin that might act against these resistant organisms and Amlaki could be an interesting candidate. *Phyllanthus emblica* (Gaertn) (syn. *Emblica officinalis*) belongs to the Family Euphorbiaceae. Phyllanthus emblica is very large genus consisting of approximately 550 to 750 species and is subdivided in to 10 or 11 subgenera. *Phyllanthus emblica* L. (syn. *Emblica officinalis*) has been extensively used, both as fruit and as tonic for its therapeutic potentials. It is highly nutritious and is rich natural source of vitamin C, carotene, thiamine, riboflavin, niacin, amino acids, and minerals such as calcium, phosphorus, iron [5]. It also contains tannins and colloidal substances, phyllembic acid, lipids, gallic acid, ellagic acid, trigalloylglucose, terchebin, corilagin and emblicol. Phyllembin and mucic acid have been isolated from the fruit pulp. Studies have shown that the active compounds contained by *P. emblica* have significant medicinal value. It has its beneficial role in treatment of diabetes, cough, asthma, bronchitis, dyspepsia, colitis, flatulence, hyper acidity, peptic ulcer, skin diseases, leprosy, inflammations, anaemia, hepatopathy, jaundice, diarrhoea, dysentery, haemorrhage, leucorrhoea, cardiac disorders, intermittent fevers and greying of hair and is given to cancer. It has anti-viral, anti-bacterial, anticancer, antiallergic, and anti-mutagenic properties6. Amlaki is proven to rejuvenate all the organ systems of the body, provide strength and wellness. To overcome antibiotic resistance, essential oils and medicinal plant extracts present alternative solutions that are safer, more efficacious and multifunctional. Amlaki consist of polyphenols, flavonoids, gallic acid, ellagic acid and other bioactive compound that possess antioxidant, antibacterial, antiseptic, antiviral and antifungal properties. Amlaki has been reported to inhibit the growth of several antibiotic resistant strains of bacteria. The present study is aimed to investigate the antibacterial activity of Amlaki

Table 1: Experiment carried out.organism [7,8].

Experiment No.	Targets
Expt-I	Antibacterial sensitivity testing of aqueous extract of *Phyllanthus emblica* (Amlaki) against *Pseudomonas aeruginosa* by using disc diffusion method.
Expt-II	Antibacterial sensitivity testing of ethanolic extract of Amlaki against test organisms by disc diffusion method.
Expt-III	Determination of Minimum Inhibitory Concentration (MIC) of aqueous extract of Amlaki against test organisms by broth dilution technique.
Expt-IV	Determination of MIC of ethanolic extract of Amlaki against test organisms by broth dilution technique.
Expt-V	Determination of MIC of ciprofloxacin against test organisms by broth dilution technique.
Expt-VI	Subculture studies of materials from effective aqueous extract, ethanolic extract and ciprofloxacin preparations for confirmations of respective results of Experiments III, IV and V.



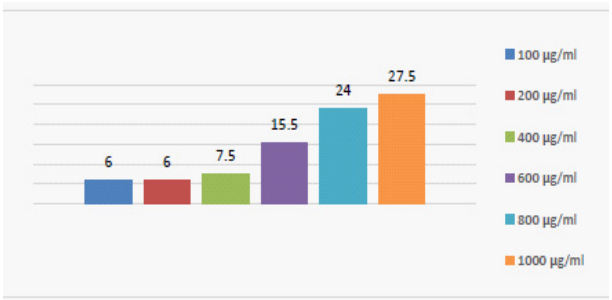


Figure 1: Zone of inhibition at different concentrations of AAE.

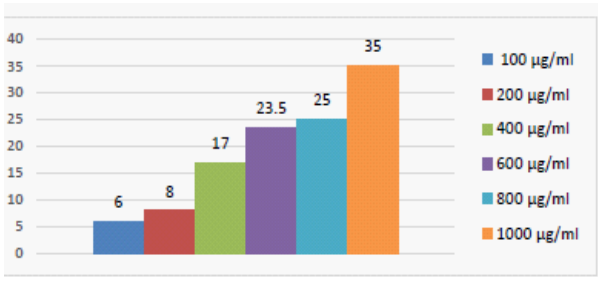


Figure 2: Zone of inhibition at different concentration of EAE.

against *Pseudomonas aeruginosa*. So that Amlaki can be used against these microorganisms in antibiotic resistance.

Method and materials

This experimental study was conducted in the Department of Pharmacology & Therapeutics in collaboration with the Department of Microbiology, Mymensingh Medical College, Mymensingh, between July 2018 to June 2019. The test organism *Pseudomonas aeruginosa*, ATCC 27853 and all the test organisms (Reference strains) were collected from Department of Microbiology of same medical College. Amlaki were bought from local market of Mymensingh. A Quinolones antibiotic (ciprofloxacin) which was bought in injectable form (200 mg/100 ml) manufactured by Opsonin Pharma Limited, Bangladesh. The whole study was fragmented into six separate experiments (Table 1). In this study antimicrobial activity of Amlaki was analysed in terms of the Zone of inhibition (ZOI) and Minimum Inhibitory Concentration (MIC) for the test organism [7,8].

Results

For AAE, the maximum Zone of Inihition (27.5 mm) was observed at 1000 µg/ml concentration. But *P. aeruginosa* started showing definite activity from 600 µg/ml where was 15.5 mm (Figure 1). Negative control (disc containing only sterile D/W) showed no zone against any bacteria. For EAE *P. aeruginosa* started showing definite activity from 400 µg/ml with ZOI of 17 mm compared to Negative control. Maximum ZOI was 35 mm at 1000 µg/ml against aforesaid bacteria (Figure 2).

The MICs of AAE was 500 µg/ml whereas for ethanolic extract of Amlaki the MIC was 300 µg/ml. In case of ciprofloxacin the MIC was 1 µg/ml. The result of expt III-IV was confirned by the subculture studies.

Discussion

In Experiment-I, Zone of Inhibition of AAE for *P. aeruginosa*, were 6 mm, 6 mm, 7.5 mm, 15.5 mm, 24 mm and 27.5 mm at difierent concentrations respectively (100, 200, 300, 400, 600, 800 µg/ml). Dharajiya et al analyzed the antimicrobial activities of *E. officinalis* fruits against different pathogenic bacteria, *E. coli, Serratia marcescens, P. aeruginosa and Bacillus cereus* by agar well diffusion method using different solvents such as aqueous, ethanol, methanol and hexane8. In aqueous extract, ZOI for *P. aeruginosa* was 12 ± 1.0 mm at 100 and 50 mg/ml which is observe closer to ZOI (15.5 mm) at 600 µg/ml in our study. Another study was carried out by Selvamohan et al to assess the antibacterial activities of *P.emblica* against pathogens including *Klebsiella pneumonia*, *S. aureus, P. aeruginosa* and *E. coli*. Three different solvents such as aqueous, ethanol, and methanol were used in this case at various concentrations (20, 30, 40, 50, 60 µl). In aqueous extract of *P. emblica* has high impact on the human pathogenic bacteria such as 22 mm, 21 mm, 21 mm and 18 mm against *Klebsiella sp., Staphylococcus sp., Pseudomonas sp*. and *E. coli* respectively [9]. In present study for *P. aeroginosa* 24 mm ZOI was seen at 800 µg/ml which somewhat coincide with the aforesaid research. In Expt-II for *P. aeruginosa*, ZOI were 6 mm, 8 mm, 17 mm, 23.5 mm, 25 mm and 35 mm at same concentrations respectively. As per Dharajiya et al. in ethanolic extract, for P. aeruginosa ZOI was 13 ± 1.0 mm [8]. But in present study it was 17 mm at 400 ug/l for the same test organism. However, Selvamohan et al found better impact (19-23 mm) against all pathogens in case of ethanol extract. However highest antimicrobial activity (ZOI:22 mm) was observed aganist *E. coli* and *Pseudomonas sp*. respectively, for the methanolic extracts of *P.emblica* [9]. In the present study, *Pseudomonas sp.* 35 mm which show dissimilarity with aforementioned study. In Expt -III, the Minimum Inhibitory concentration (MIC) of aqueous extract of Amlaki (AAE) was determined by broth dilution technique against the test organisms. In present study the MICs of aqueous *P. emblica extract for P aeruginosa* 500 µg/ml. Dharajiya et al [8] determined the MIC of aqueous extract of *P. emblica* by the dilution method against pathogenic organisms including *P. aeruginosa, E. coli* and in that study the MIC for, *P. aeruginosa* it was 25 mg/ml (or 25000 µg/ml) which is way apart from that of the current study. This dissimilarity might be due to different technical procedure.

In Expt -IV, the MIC of ethanolic extract of Amlaki (EAE) was determined by broth dilution technique against the test organisms. Nahor et al determined the MIC of ethanolic extract of Amlaki (EAE) by the dilution method against pathogenic organisms including *S. aureus, P. aeruginosa, E. coli*, and *K. pneumoniae*. In that study the MIC, for *P. aeruginosa* was 25.0 µg/ml which is again observed to be inconsistent with our findings which can be explained by the different extract and different technical procedure [10].

Conclusion

Our study suggests that both aqueous and ethanolic extracts of Amlaki have antibacterial effect against *P. aeruginosa*. Effect of ethanolic extract was more antimicrobial effect against *P. aeruginosa.* However, the MIC for EAE is almost half than that for AAE. Further study is required to isolate the active compounds responsible for the antibacterial activity of Amlaki and also to know their efficacy, mechanism of action, toxicity profile and safety margin for its therapeutic use. Hopefully, that would lead to the discovery of new and more potent antimicrobial agents originated from Amlaki.

References

1. Kumar, Anil, Singh A. & Dora J. Essentials perspectives for Emblica officinalis." International journal of pharmaceutical and chemical sciences. 2012; 1: 11-18.
2. Yebpella GG, Adeyemi Hassan MM, Hammuel C, Magomya AM, Agbaji AS and Okonkwo EM. Phtyochemical screening and comparative study of antimicrobial activity of Aloe vera various extracts. Afri. J. Microb. Res. 2011; 5(10): 1182-1187.
3. Kedarnath NK, Surekha, Ramesh S, Mahantesh SP and Patil CS. Phytochemical Screening and Antimicrobial Activity of Aloe vera L. W. Res. J. Med & Aromat. Pl. 2012; 1(1): 11-13.
4. Mukherjee PK, Wahile A. Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. J Ethnopharmacol. 2006; 103: 25-35
5. Dhale DA. Pharmacognistic evaluation of phyllanthus embillica linn . Inter.J. Pharma and biosciences. 2012; 3(3): 210-7.

6. Nair R, Sumitra V, Chanda C. Antibacterial activities of some medicinal plants of western regions of India. Turkish Journal of Biology. 2007; 3: 231-236.

7. Ghani A. Medicinal Plant of Bangladesh with Chemical Constituents and Uses. 2nd ed. Asiatic Society of Bangladesh,Dhaka. 2003; 71-72.

8. Dharajiya D, Patel P, Moitra N. Antibacterial activity of Emblicaofficinalis (Gaertn.) Fruits and Vitexnegundo (L.) Leaves. Biotechnology and Pharmacy. 2015; 9: 357-368.

9. Selvamohan T, Ramadas V, Kishore SSS. Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria. Advances in Applied Science Research. 2012; 3: 3374-3381.

10. Nahor U, Ahmed Z. Antimicrobial Activity of PhyllanthusEmblicaand Allium Sativum: Comparative Analysis of Antimicrobial Action of CrudeandEthanolic Extract of These Natural Plant Products. IOSR Journal of Pharmacy and Biological Sciences. 2012; 4: 21-26.