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Determination and comparison of antimicrobial activity of *Psidium guajava* and *Emblca officinalis* against MDR bacteria

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Abstract

The antimicrobial activities of *Psidium guajava* (guava) and *Emblca officinalis* (amla) were determined and compared against 2 gram +ve (*Staphylococcus aureus* and *Bacillus cereus*) and 2 gram -ve bacteria (*Salmonella typhi* and *Escherichia coli*), which are Multi Drug Resistant (MDR). The guava and amla leaves were crushed and their extract was taken in methanol and ethanol respectively. The efficiency of these extracts were tested against MDR bacteria through well diffusion assay. In this study both extract showed inhibitory activity against MDR bacteria. The methanol extract of guava showed maximum antimicrobial activity against *B. cereus* (gram +ve) followed by *S. aureus* (gram +ve) while lesser inhibition against *S. typhi* (gram -ve) and least inhibition against *E. coli* (gram -ve) was observed. The ethanol extract of amla showed maximum inhibition against *Salmonella typhi* (gram -ve) while minimum against *Staphylococcus aureus* (gram +ve). On the basis of present findings it was concluded that both the extracts possesses antimicrobial and pharmacological properties, hence can be used parallel to synthetic drugs which have undesirable side effects.

Keywords: *Psidium guajava*, *Emblca officinalis*, methanol and ethanol extract, antimicrobial activity MDR pathogens

Introduction

Although this era witnesses amazing success in the development of technology, science, medicine and the discovery of antibiotics and making use of them as chemotherapeutic agents. This has made the medical fraternity to believe that they will eradicate various infectious diseases but to some extent we failed to control the dramatic spread of infectious diseases. As per the WHO reports, more than 80 % of the world's population relies on traditional medicine for their primary healthcare needs. The struggle between man and microbes began since their appearance on earth. After the development of first antibiotic 'Penicillin' by Alexander Fleming (1929) interest in this magic drug antibiotics increased, leading to new waves of synthetic antibiotics. Antibiotics are chemical substances produced from various microorganisms (bacteria, virus, fungi) that kill or suppress the growth of microorganisms. The misuse of antibiotics by humans, the employment of antibiotics in veterinary practices and the growing presence of antibiotics in water, soil, food contribute to the problem of antibiotic resistance, leading to prevalence of MDR infections. Hence the emergence of MDR strains of different groups of microorganisms has become a major cause of failure of the treatment of infectious diseases. To this emerging problem of antibiotic resistance, phytochemicals obtained from medicinal plants may be one of the remedy of this problem. This further drives the need to screen medicinal plants for novel bioactive compounds as plant based drugs are biodegradable, safe and almost t no side effects (Ramya *et al*, 2008) [9]. The green medicines are widely believed as safe in contrast with expensive synthetic drugs (antibiotics) that have undesirable side effects along with beneficial effects (Alviano *et al.*, 2009). In the past few decades, the curiosity to evaluate plants possessing antimicrobial, antifungal, anti-inflammatory activity for various diseases has grown manifold and a large number of biologically active compounds have been characterized. The WHO is promoting and facilitating the effective use of herbal medicine for the health program of developing countries. Plants are the largest biochemical and pharmaceutical stores on our planet. These living stores are able to generate endless biochemical components. Medicinal plants are very rich in many variety of secondary metabolites of antimicrobial properties such as saponins, tannins, alkaloids, alkenyl phenols, glycoalkaloids, etc. The present study was undertaken to evaluate and compare the antimicrobial activity of methanolic extract of guava leaves and ethanolic extract of amla leaves on 4 MDR bacteria namely, *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli*. *Psidium guajava* (guava) belonging to family myrtaceae

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is an important food crop and medicinal plant in tropical and subtropical countries. Guava leaves have many therapeutic properties and offer an array of health benefits. Being packed with antioxidants, antibacterial and anti-inflammatory agents guava leaves are considered natural pain reliever. The chemicals contained in the leaves of these plants such as polyphenols, carotenoids, flavonoids and tannins can be very effective in treating various diseases. Guava leaves are beneficial in diabetes, diarrhoea, dysentery, dengue, throat diseases, etc. *Embllica officinalis* (Amla) had been used to treat and manage disease since the ancient times. It prevents cancer due to the presence of some important compounds such as polyphenols. It is the reservoir of minerals, vitamins and other bio chemical substances. Leaves are beneficial in diabetes, lowers cholesterol, in diarrhoea, dysentery, indigestion, throat diseases, dengue fever, etc. In the light of above mentioned fact, the present study was carried out to evaluate antimicrobial activity of above mentioned plant parts against selected GIT pathogens.

Materials and Methods

1. Place of Work: The present study was carried out in Centre for Microbiology, Department of Botany, Ewing Christian College, Allahabad.

2. Study Samples: The medicinal plants used for evaluating their antimicrobial (antibacterial) activity were *Psidium guajava* (Safeda variety) and *Embllica officinalis*. The plant parts used for study were young fresh leaves.

3. Collection of plant samples: Fresh leaves of guava and amla plant were collected from Khusrau Bagh, Allahabad. Leaves from 3 different plants of same variety were collected in separate zip bags and marked as Sample 1, Sample 2, and Sample 3. This way 3 samples of each guava and amla leaves were collected. Leaves were properly examined and those attacked by insects were removed.

4. Preparation of Extracts: Leaf samples were properly washed and dried in ovens. These were then grounded into fine powder using mortar and pestle. This powder was then used for extract preparation with solvents (methanol and ethanol).

Methanolic extract of guava leaves: 5g of guava leaf powder was mixed with 25ml of methanol in conical flask. This flask was kept in rotatory shakers at 150rpm for 24 hours. After 24 hours it was filtered and then solvent was evaporated (Mishra and Babele, 2014) [6]. Similarly, extracts of all the three samples were prepared and stored in sample bottles marked as S1, S2 & S3.

Ethanol extract of amla leaves: For the preparation of ethanol extract of amla leaves, 5g of amla leaf powder was mixed with 50ml of ethanol (70%) and soaked for 24 hours. This mixture was then filtered by a muslin cloth and kept for evaporation at room temperature (Gupta and Ramteke, 2011)

[8]. These extracts were then stored in sample bottles and marked as S1, S2 & S3.

5. Bacterial Strains / Test organisms: The antibacterial activity of methanol and ethanol extracts of guava and amla respectively was evaluated against 2 gram +ve bacteria, viz., *Staphylococcus aureus* and *Bacillus cereus*, and 2 gram -ve bacteria, viz., *Escherichia coli* and *Salmonella typhi*.

6. Inoculum preparation: Nutrient broth was prepared and autoclaved for 15-20 minutes and was transferred into test tubes (LML each). It was then used for preparing bacterial suspensions which was incubated for approximately 24 hours and used for experiment.

7. Antimicrobial assay: The plant extracts were tested on Mueller Hinton Agar medium (MHA) to detect their antimicrobial activity against *S. aureus*, *S. typhi*, *B. cereus* and *E. coli* through well-diffusion assay. This whole work was carried out in laminar air flow chamber. The MHA media was poured on all the petriplates. One MHA plate was marked as media control plate and kept aside (sealed). When the media solidified in other plates, they were streaked/inoculated with bacteria. Sterile cotton swab was dipped into bacterial suspension, rotated several times and pressed firmly on the inside wall of the tube above the fluid level removing excess inoculum. The surface of agar plate was streaked over by swab, while rotating the plate to ensure an even distribution of inoculums with final swab around the rim. The plates were then closed and kept inverted. Out of all the plates, 4 inoculated petriplates were marked as organism control plates and sealed. In other petriplates, wells were made by sterile metallic borer with 5mm diameter. The wells were arranged in triangle formation in each plate and were marked as S1, S2 & S3. In 2 plates, only 1 well was made. In the wells, methanolic extract of guava (3 samples) and ethanolic extract of amla (3 samples) were poured with the help of micropipette (0.5ml). Each tip was used only once. This way 4 plates of methanol extract of guava and 4 plates of ethanol extract of amla were prepared. Plates were sealed properly. In the plates having only 1 well, the solvents used for extraction, i.e, methanol and ethanol (70%) were poured. These plates were marked as treatment control plates. All the plates were kept for incubation for at least 24 hours. After 24 hours each plate was examined for inhibition zone.

Result and Discussion

The results indicated that extracts of both, methanol and ethanol extracts of guava and amla leaves respectively showed inhibitory or antimicrobial activity against MDR bacteria, viz., *S. typhi*, *S. aureus*, *B. cereus* and *E. coli*.

The diameter of zone of inhibition for each sample was recorded against each MDR and 5mm (borer diameter) was reduced from the measured diameter of zone of inhibition and then mean of ZOI values was calculated and recorded in table no. 1.

Table 1: Showing the mean zone of inhibition obtained by methanol and ethanol extract against MDR bacteria.

Plant	Mean zone of inhibition (in mm) / Test organism			
	<i>S. aureus</i>	<i>S. typhi</i>	<i>B. cereus</i>	<i>E. coli</i>
Guava leaf (Methanol)	18.22	6.77	18.77	4.11
Amla leaf (Ethanol)	12.99	23.77	22.33	17.44

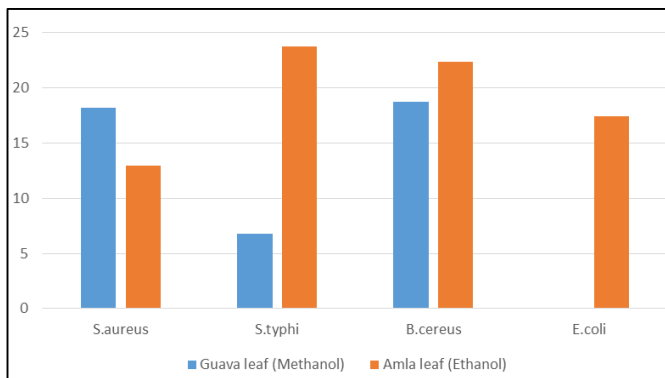


Fig 1: Antimicrobial activity of plant extracts against bacterial pathogens

Psidium guajava (guava) plant extract (leaves) showed the highest antimicrobial activity in methanol against *B.cereus* (Gram +ve), the mean zone of inhibition (ZOI) being 18.77mm. The lowest antimicrobial activity of guava in methanol extract was observed against *E. coli* with mean ZOI being 4.11mm. Clear zone of inhibition for *E. coli* was not obtained in 24 hours but took 48 hours to show inhibition. The inhibitory activity should decrease with time but in this it showed significant increase. In few samples of methanol extract of guava no inhibition was obtained against *E. coli*. The resistance of Gram -ve bacteria could be due to its cell wall structure which is biochemically more complex than Gram +ve and appears usually trilayered, besides peptidoglycan, there are phospholipids, proteins, and lipopolysaccharides in the cell wall.



Plate 1: Antimicrobial test of guava extract against *S. aureus*

Emblica officinalis (Amla) plant extract (leaves) showed the highest antimicrobial activity against *S. typhi* (Gram -ve) with mean ZOI being 23.77mm. The lowest antimicrobial activity of amla in ethanol extract was observed against *S. aureus* (Gram +ve) with mean ZOI being 12.99mm. The reason may be the presence of chebullic acid, gallic acid, saponins, etc. whose nature enhance in presence of ethanol. Hence, ethanol extract showed antimicrobial activity even against Gram -ve MDR bacteria. Nascimento *et al.* (2000) [7] conducted study which supports the finding of present study in which guava extract showed inhibitory effects against *S. aureus* and *B. cereus* but no effect on *E. coli* and *S. typhi*.



Plate 2: Antimicrobial test of amla extract against *S. typhi*



Plate 3: Antimicrobial test of amla extract against *E. coli*

Biswas *et al* (2013) [3] also conducted study which supports this study, showing the inhibitory activity of guava leaves against Gram +ve but Gram -ve bacteria were resistant to the extract whereas Kaneria *et al* (2013) [5], oppose the findings of concurring Gram -ve bacteria. Mishra and Babele (2014) [6], through their study observed 14mm zone of inhibition against *S. aureus*, while in this present study, mean zone of inhibition of *S. aureus* was observed to be 18.22 mm. Gupta and Ramteke (2011) [8] observed highest antimicrobial activity by the ethanolic extract of amla against *E. coli* producing zone of inhibition 29mm, while in present study, ethanolic extract of amla showed highest antimicrobial activity against *S. typhi* with ZOI 23.77mm. Esimone *et al* (2012) [4] opposes the present study by obtaining zone of inhibition by aqueous and methanol extract against MRSA is elates ranging from 5-20 mm but in the present study, zone of inhibition obtained by methanolic extract of guava against *S. aureus* was 18.22mm. Vijayalakshmi *et al* (2007) [10] in their study recorded the maximum zone of inhibition produced by the methanol extract of *Emblica officinalis* against *E.coli* and *Staphylococcus aureus* in the present study, maximum mean zone of inhibition (ZOI) was obtained against *S. typhi* while minimum ZOI against *S.aureus* (12.99mm) and *E. coli* (17.44mm). According to the study conducted by Gautam and Shukla (2017) [2], highest zone of inhibition was observed for the 5% aqueous extract against *Bacillus* (34mm) followed by *E. coli* (24mm). In the present study, highest zone of

inhibition obtained by ethanol extract of amla was against *S. typhi* (23.77mm) followed by *B.cereus* (22.33mm). On the basis of the study this may be concluded that both the methanolic and ethanolic extracts showed antimicrobial activity against all the MDRs, however Methanolic extract showed highest antimicrobial activity against *B.cereus* while least against *E. coli*. Further it was also observed that Gram -ve bacteria showed resistance to an extent towards the inhibitory activity of methanol extract of guava. The main reason behind this resistance is may be the biochemically complex structure of cell wall which is trilayered, consisting of phospholipids and lipopolysaccharide besides peptidoglycan. The least inhibitory activity might be due to the degradation of cross-linkages of peptidoglycan. The ethanolic extract comparatively showed more inhibitory or antimicrobial activity against MDRs.

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