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Antimicrobial activity of methanolic extract of langra mango pulp

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Abstract
Mango is antioxidant rich one of the most popular fruits in the world. Mangoes (*Mangifera indica*) belong to genus Mangifera under the family of Anacardiaceae. In Bangladesh, Mango is called as the "King of fruit" over a large area, but locally known as "aam". Mango contains different types of chemicals and most of them are bioactive compounds such as polyphenols, vitamin C, carotenoids, anthocyanin, and flavonoids. The present study was to extract the chemicals from Langra mango as well as investigated the antimicrobial properties by using various *in vitro* methods. The mature fruit was extracted with methanol. Chemicals from mango pulps were extracted by using Rotary evaporator. Phytochemical tests were performed to investigate the possible phytochemicals present in the mango pulp extract. Antimicrobial activity and Minimum Inhibitory Concentration (MIC) were performed by disc diffusion and broth dilution assay against five (5) pathogenic bacteria. The antimicrobial activity exerted by the extract was compared with standard and results showed that extract contained significant antimicrobial activity. The order of zone of inhibition was *Staphylococcus aureus (17mm)* > *Bacillus subtilis* (16 mm) > *Escherichia coli* (14 mm) > *Salmonella typhi (14 mm)* > *Klebsiella pneumoniae* (12 mm). The MIC values were in the range 0.6 mg/ml to 1.25 mg/ml for extract of Langra mango pulp. The lowest MIC was found for *Escherichia coli* (0.6 mg/ml). So the extract (methanolic) of langra mango pulp has potent antimicrobial properties.

Keywords: Langra mango pulp, Antimicrobial activity, Minimum Inhibitory Concentration (MIC), Pathogenic bacteria

Introduction
Medicinal plants are potential sources of drugs and of therapeutic importance because these plants have abundant secondary metabolites. This is why using of plant extracts as therapeutic agents is increasing day by day from ancient time [1, 2]. Because of natural origin and fewer side effects, Herbal medicine and these drugs are gaining acceptability and popularity day by day [3]. Beside the source of micronutrients, vitamins and other phytochemicals, Mango fruits are also provide energy, dietary fibres, carbohydrates, proteins, fats and phenolic compounds [4]. All components are vital not only for normal human growth but also for development and health [5]. Both unripe and ripe fruits have verity of ethnomedicinal use and effective against different diseases. The unripe fruits are acidic, digestive and carminative and useful against dysentery ophthalmia, eruptions, urethrorrhoea and vaginopathy. The ripe fruits are used against anorexia, dyspepsia, cardiopathy, haemoptysis, haemorrhages from uterus, lungs and intestine, emaciation, and anemia because they are refrigerant, sweet, emollient, laxative, cardiotonic, haemostatic, aphrodisiac, and tonic. Mango extract contain gallotannin and mangiferin. Presenting of these chemicals, extracts shows antibacterial activity against both Gram-positive and Gram-negative bacteria [6, 7, 8, 9]. Mangiferin (Mangiferin is a xanthonoid found in mangoes) is able to interfere with the assembly or functioning of microtubule filaments or cellular matrix components or inhibition of the telomerase and the gene. Another possible mechanism is the enhancement of the cellular apoptosis [10, 11, 12, 13, 14]. Hence the major objective of the present work was to evaluate the chemical composition, antibacterial effect activity of ripe mango pulp extract.

Materials and Methods
According to the investigational procedure the mature fruit mango were collected from Mohakhali Kacha bazar, Dhaka, Bangladesh in the month of May, 2016.

Preparation of extract
The pulp parts of the collected mango fruits were shade dried for seven days.

After drying pulp (50 gm) were dissolved in methanol (250 ml methanol) for five days in a glass container (appropriate labeling) with occasional shaking. The mixtures were filtered through a plug of cotton and separate the excess solvent by using rotary evaporator. After separation remaining portion was used as a sample [15].

Microorganisms

Pure culture of *Escherichia Coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Staphylococcus aureus* were obtained from the Microbiology Laboratory of Department of Pharmacy, Primeasia University, Dhaka, Bangladesh (10^6 CFU ml-1).

Phytochemical Screening

Carbohydrates, proteins, alkaloids, glycosides, anthraquinone glycosides, tannins, saponins, flavonoids and resins were detected by standard procedures. Different reagents were (Molishch's reagents (10% naphthol in alcohol) - for carbohydrate test, Aqueous sodium hydroxide solution- for glycoside test, Dilute sulphuric acid and NaOH solution- for glycoside test, Fehling's solution- for glycoside test, 10% Ammonia solution- for anthraquinone glycoside test, Mayer's reagent (potassiomercuric iodide solution), Wagner's reagent (solution of I in KI), Hager's reagent (Saturated solution of picric acid), Dragendroff's reagent (Bismuth sub nitrate and acetic acid solution)- All for alkaloid tests, Conc. Hydroclric acid – for flavanoid test, Conc. Sulphuric acid- for steroid test, $FeCl_3$ (5%) - for tannin test) used for this purposes [15].

Antimicrobial Activity

Antimicrobial activity of the fruits extracts (Langra mango) was tested by disc diffusion method. The extract solution was prepared at a concentration of 100 µg/disc. Then extract was infused into the paper discs and placed onto the nutrient agar media inoculated with the test bacteria and incubated at 37° C for 24 h. Kanamycin (30 µg/disc) (oxoid, UK) was used as standard. After incubation, zone of inhibition formed around the disc was examined and assessing the antimicrobial activity on the basis of measurement of diameter of the inhibited zone formed around the disc [16].

Minimum Inhibitory Concentration (MIC)

Determination of the Minimum Inhibitory Concentration (MIC) was carried using the broth dilution method. To obtain a concentration 100 mg/ml, 50µl/4ml of the reconstituted extract solution at a concentration of 10mg/ml was added to test tube containing 1 ml of sterile broth. 1 ml of this dilution was transferred to another test tube till the 10^{th} test tube was reached. The 11^{th} test tube did not contain any extract, but a solution of pure solvent served as negative control.

Then 1 ml of an 18 h old culture of each of the organisms earlier adjusted at 10^6 CFU/ml was put into each tube and thoroughly vortexes. The tubes were incubated at 37° C for 24 h and observed the growth of bacteria in form of turbidity. The test tube with the lowest dilution with no detectable growth by visual inspection was considered the MIC [16].

Result

Table 1 shows the results of Phytochemical analysis of the methanolic extract of Langra mango pulp and showed that all critically important secondary metabolites were present in the langra mango pulp extract. Antibiotic sensitivity test was performed against five strains. The results are shown in Table 2, Fig: 1. All five tested bacteria showed sensitivity on the extract at different dimension. Results of Minimum Inhibitory Concentration (MIC) of the methanolic extract of Langra mango is given in table 3 and impressive result was shown against *E.coli* bacteria.

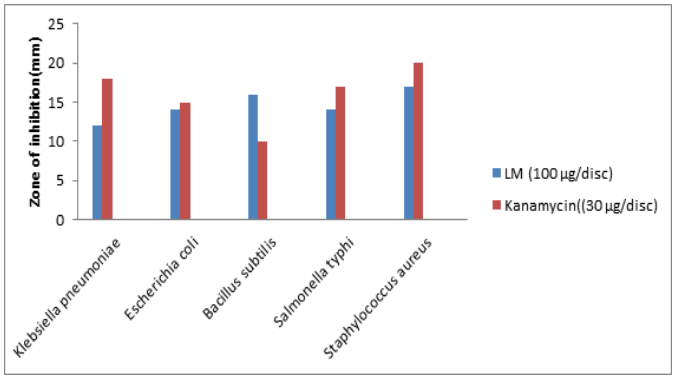

Fig 1: Antibiotic sensitivity test against five strains

Table 1: Results of Phytochemical analysis of Langra mango

Tests	Results
Carbohydrates	present
Glycosides	present
Saponins	present
Flavonoids	present
Tannins	present
Resins	present
Proteins	present
Alkaloids	present

Discussion

Fruits and vegetables are considered the main food source for some essential nutrients and also include a series of bioactive components, which might have multiple effects in the fields of health [17]. Mango is considered a very useful remedy and energizer in Ayurveda. The insoluble fibers, present in mangoes give laxative effect that helps the elimination of waste from the colon and prevents constipation. Extracts of leaves, bark, stem and unripe mangoes are believed to possess antibacterial properties against some micro-organisms. Antioxidants are playing critical role for preventing cancer. Mango fruits contain antioxidants and enzymes and both are playing an important role in the prevention/protection of different cancer (colon, breast, leukemia and prostate) and heart disease. Serum cholesterol regulating components such as fiber, pectin and vitamin C are also present in the mango fruits. Some of the flavonoids present in the fruit are believed to strengthen the immune system of human body. Both fiber and enzymes makes mangoes favorite for healthy digestion [18]. Based on this aspect the present study aims to analyze the antimicrobial activity of methanol extract of the Langra mango pulps. Phytochemical screening on this fruit reported the presence of plant secondary metabolites alkaloids, tannins, flavonoids and glycosides in the pulp extract of this fruit (table 1). The antimicrobial activity of methanol extract of Langra mango pulp against five (5) bacteria was shown in Table 2. Kanamycin (30µg/disc) was used as standard. The result showed that the zone of inhibition observed by the extract against the tested bacteria was significant compared to the standard. From the table 2 it is shown that the highest zone of inhibition observed by the langra mango extract was 17 mm (standard 20 mm) for *Staphylococcus aureus* and the lowest inhibition zone is 12 mm (standard 18 mm) for *Klebsiella pneumonia.* No zone of inhibition was observed for

negative control which may be correlated with their any involvement in the antimicrobial activity of the extracts. MIC results also supported the antimicrobial claim as very low concentration of the extract was effective against the tested bacterial strains to inhibit their growth table 3 and also suggest that this pulp part of this fruits possesses strong antimicrobial activity. The presence of alkaloids in plant extract may contribute to the antibacterial activity [16]. Antibacterial activity of plant extract may also be exerted due to the presence of plant secondary metabolites such as flavonoids [19].

Table 2: Results of antibiotic sensitivity test against five strains

Test organisms	Zone of inhibition (mm)	
	LM (100 µg/disc)	Kanamycin((30 µg/disc)
Klebsiella pneumoniae	12	18
Escherichia coli	14	15
Bacillus subtilis	16	10
Salmonella typhi	14	17
Staphylococcus aureus	17	20

LM: Langra mango

Table 3: Results of MIC against five strains

Test organisms	LM (mg/ml)
Klebsiella pneumoniae	1.25
Escherichia coli	0.6
Bacillus subtilis	1.25
Salmonella typhi	1.25
Staphylococcus aureus	1.25

LM: Langra mango

Conclusion

The findings of our present work emphasizing on the antimicrobial activity of the pulp of Langra mango shows significant activity against five microorganisms. This work can be authenticated by isolating and exploring the responsible chemical constituents for the given activities. This could lead to the discovery of some novel molecules in this particular field of interest.

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