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Antimicrobial activities of *Psidium guajava, Carica papaya* and *Mangifera indica* against some gram positive and gram negative bacteria.

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Abstract
The advent of science to the search for antibiotics principally depends on medicinal plants as raw materials. This in-vitro study corroborated the antimicrobial activity of the plants used mostly in folklore medicine. The extract of leaves and barks of *Psidium guajava*, of *Mangifera indica*'s leaves and fruits and seeds of *Carica papaya* were tested against *Bacillus cereus, Bacillus subtilis, Escherichia coli* and *Salmonella typhi*. All extracts (except *P. guajava* bark) showed activity against those isolates but were dose dependent. The extract of *P. guajava* leaves showed the highest activity against *B. cereus*, *B. subtilis* and *E. coli* while *M. Indica*'s leaves extract was effective against *S. typhi*. Both fruit and seed extracts of *C. papaya* were more active against gram positives than gram negatives. The antibacterial activities of the plant extracts were compared to the standard disks of Streptomycin. These plants could be a potential candidate for the development of new strategies to treat bacterial infections.

Keywords: Antimicrobial activity, *Psidium guajava, Carica papaya, Mangifera indica*, extract, bacteria.

1. Introduction
The era of antibiotic chemotherapy regressed approximately in the early 1940s. The discovery of Penicillin was a key breakthrough in the field of pathology. Antimicrobial is a substance that acts to inhibit the growth of harmful microorganisms or acts to destroy them, such as bacteria, virus, fungi, and protozoa. The discovery and development of antibiotics are among the most influential and successful achievements of modern science and technology for the control of infectious diseases. However, the rate of resistance of pathogenic microorganisms to conventionally used anti-microbial agents is increasing with an alarming frequency [1, 2, 3]. Surveys have revealed that almost no group of antibiotics has been introduced to which resistance had not been observed [4]. Although tens of thousands of antimicrobial compounds exist, the ability of microbes to develop resistance to even the most powerful antimicrobial compounds is amazingly rapid [5].
In addition to this problem, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often associated with different side effects on the host, which include hypersensitivity, depletion of beneficial microorganisms, and immunosuppression [6]. The number of multi-drug resistant microbial strains and the appearance of strains with decreased susceptibility to antibiotics are continuously increasing.
This situation forced scientists to search for new or antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective infection fighting strategies. Therefore, there is a need to develop alternative therapeutic agents for the treatment of infectious diseases from medicinal plants.
However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy while there are some advantages of using medicinal plants, such as often fewer side effects, better patient tolerance, relatively affordable treatment, profound therapeutic benefit, acceptance due to long history of use and being renewable in nature. For these reasons, researchers are increasingly turning their concentration to herbal products, looking for new leads to develop better drugs against multiple drug resistant microbial strains. Herbal medicine is still the stronghold of about 75-80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents [7].
A medicinal plant is any plant in which one or more of its organs contain substances that can

be used for therapeutic purposes that are precursors for synthesis of valuable drugs. In the recent years, research on medicinal plants has drawn a lot of attentions globally for its versatile applications. Medicinal plants are the wealthiest bio-resources of drugs of traditional system of medicines, modern medicines, food supplements, nutraceuticals, folk medicines, pharmaceutical intermediates and chemical activities for synthetic drugs. Scientific experiments on the antimicrobial properties of plants and their constituents have been documented in the late 19th century [8]. Large body of evidence has been gathered to demonstrate the promising potential of medicinal plants used in various traditional, complementary and alternate systems of treatment of human diseases. Traditionally used medicinal plants produce different compounds of identified therapeutic properties. This revitalization of interest in plant derived drugs is mainly due to the current extensive belief that, "green medicine is safe".

Many works have been done which aim at knowing the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of microbial infections (both topical and systemic applications) as possible alternatives to chemically synthesized drugs to which many infectious microorganisms have become resistant. During the last ten years the pace of development of new antimicrobial drugs has slowed down while the frequency of resistance (especially multiple) has increased astronomically [9].

For a long period of time, plants have been precious sources of natural remedy for maintaining human health, especially in the last decade, with more intensive studies for natural therapies.

Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made immense contributions to human health and well being. Plants contain numerous biologically active compounds, many of which have antimicrobial properties [10]. Numerous studies have been performed with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds [11, 12, 13].

2. Materials and Methods

2.1 Collection of plant materials

Leaves and barks of *Psidium guajava* L., leaves of *Mangifera indica* L., seeds and fruits of *Carica papaya* L. were collected from local area of Chowgacha, Jessore, Bangladesh.

2.2 Microorganisms

The antimicrobial activity of different plant extracts were tested against four strains of bacteria. They were *Escherichia coli* (ATCC 25923), *Salmonella typhi* (ATCC 14028), *Bacillus cereus* (ATCC 11778), and *Bacillus subtilis* (ATCC 11774). These bacterial species were collected from the Microbiology Laboratory of Jessore University of Science and Technology, Jessore, Bangladesh.

2.3 Preparation of plant extracts

The collected plant parts were separated from undesirable materials and were washed with distilled water. They were sun-dried for one week and grinded into fine powder with the help of a grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced. The bioactive components were extracted according to the methods of Pandey *et al*. (2011) [14] with slight modification. The powered materials were dissolved in 80% methanol (1:10); 1 g sample should be dissolved in 10 ml of solvent [14]. Mixtures were kept in sterilized beakers wrapped with aluminium foil to avoid evaporation and exposure to light. The beakers were then kept in dark or 3 days at room temperature accompanying occasional shaking and stirring. After 3 days, mixtures were filtered through Whatman no. 1 filter paper. The filtrates obtained were concentrated using a water bath.

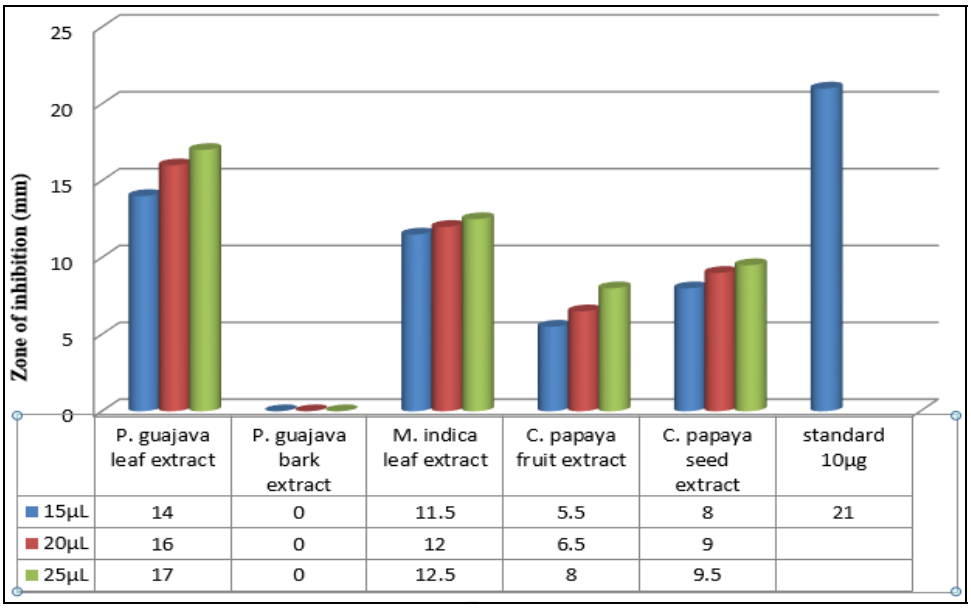
2.4 Determination of antimicrobial activity

Bacterial inoculums were prepared by Clinical and Laboratory Standards Institute (CLSI) guideline. Bacterial cultures were emulsified in normal saline and turbidity was matched with 0.5 McFarland turbidity standards. The agar disk diffusion method was followed to investigate the antimicrobial activity of plant extracts [15]. 0.1 ml of TSB broth culture of the test organisms were firmly seeded over the Mueller-Hinton agar (Lab M, UK) plates. Then paper disks soaked in crude extracts of different concentration of plant extracts were placed on the surface of agar using sterile forceps. The culture plates were kept at low temperature (4 °C) for 2-4 hours and incubated at 37 °C for 24 hours. After the incubation period formation of zones around the disks, confirms the antimicrobial activity of the respective extracts. All the results were compared with the standard antibiotic disk Streptomycin (10 µg) [16].

3. Results and Discussion

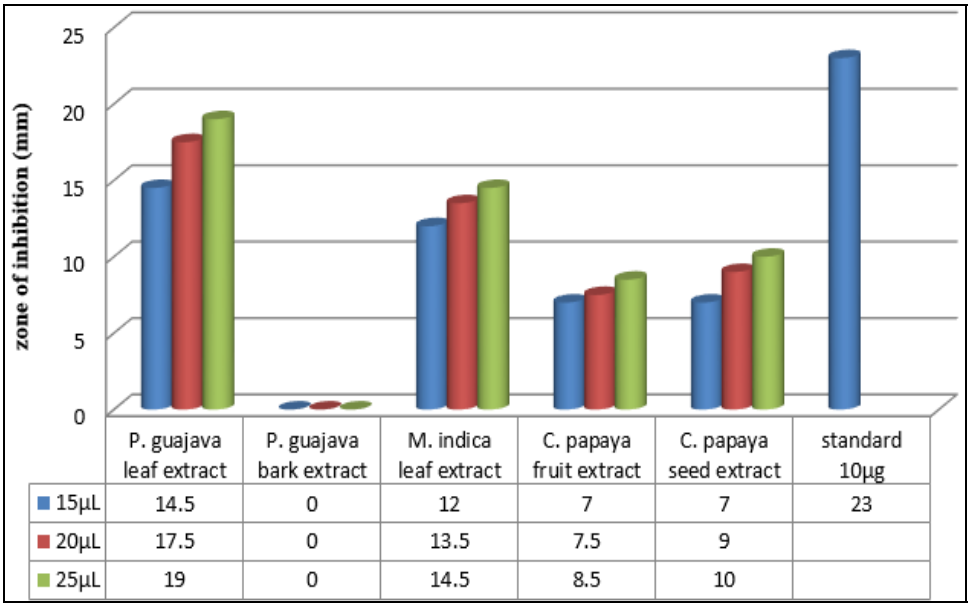
The increased frequency of resistance to commonly used antibiotics has led to the search for newer, cheap, and easily affordable drugs in the management of infectious diseases. Although conventional drugs are popular, however, herbal medicine continued to be practiced due to richness of certain plants in varieties of secondary metabolites such as alkaloids, flavonoids, tannins, and terpenoids which have been reported to have antibacterial activities [10, 17, 18].

In the present study, different concentrations of the methanolic extracts of the leaves and barks of *Psidium guajava*, leaves of *Mangifera indica* and fruits and seeds of *Carica papaya* showed antimicrobial activities against all the isolates of bacteria (*Bacillus cereus, Bacillus subtilis, Escherichia coli* and *Salmonella typhi*). The total findings are expressed in the bar charts with statistics given below (**Fig 1-4**):



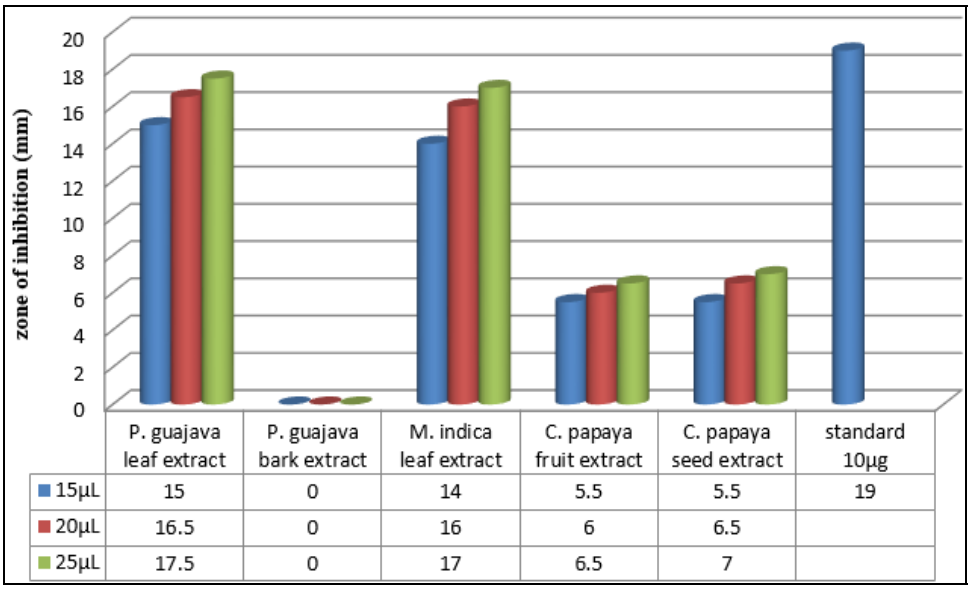
	P. guajava leaf extract	P. guajava bark extract	M. indica leaf extract	C. papaya fruit extract	C. papaya seed extract	standard 10µg
15µL	14	0	11.5	5.5	8	21
20µL	16	0	12	6.5	9	
25µL	17	0	12.5	8	9.5	

Fig 1: Statistical representation of the activity of different plant extracts against *Bacillus cereus.*



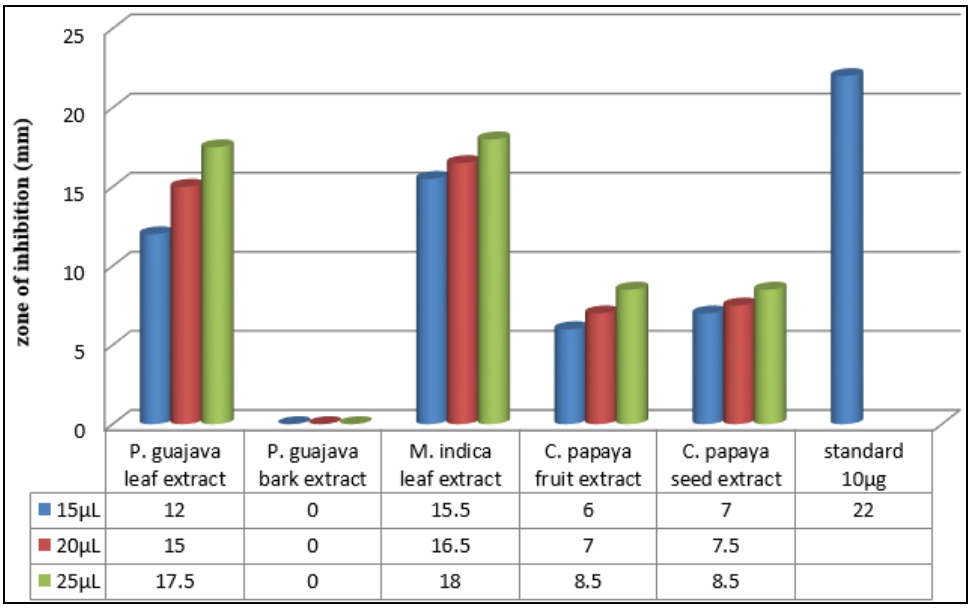
	P. guajava leaf extract	P. guajava bark extract	M. indica leaf extract	C. papaya fruit extract	C. papaya seed extract	standard 10µg
15µL	14.5	0	12	7	7	23
20µL	17.5	0	13.5	7.5	9	
25µL	19	0	14.5	8.5	10	

Fig 2: Statistical representation of the activity of different plant extracts against *Bacillus subtilis.*



	P. guajava leaf extract	P. guajava bark extract	M. indica leaf extract	C. papaya fruit extract	C. papaya seed extract	standard 10µg
15µL	15	0	14	5.5	5.5	19
20µL	16.5	0	16	6	6.5	
25µL	17.5	0	17	6.5	7	

Fig 3: Statistical representation of the activity of different plant extracts against *Escherichia coli.*



	P. guajava leaf extract	P. guajava bark extract	M. indica leaf extract	C. papaya fruit extract	C. papaya seed extract	standard 10µg
15µL	12	0	15.5	6	7	22
20µL	15	0	16.5	7	7.5	
25µL	17.5	0	18	8.5	8.5	

Fig 4: Statistical representation of the activity of different plant extracts against *Salmonella typhi.*

Among the plant extracts, *P. guajava* leaf extract showed a higher activity on all the three isolates except *S. typhi*. Higher antibacterial activity against *S. typhi* was obtained by the leaf extract of *M. indica*. Both *P. guajava* and *M. indica* leaf extracts showed significant inhibitory activities as compared to *C. papaya* fruit and seed extracts. The presence of phytoconstituents in the leaf extracts may be responsible for the antibacterial activity of the plant [19]. Reports showed that *P. guajava* contains alkaloids, phenols and tannins and *M. indica* contains tannins, glycosides; this might be the reason for their higher antimicrobial activity [20, 21].

In this study *P. guajava* bark extract showed no activity against all the four bacterial isolates at any of the concentration of the extract used, that means the bacteria are resistant to *P. guajava* bark extract. The difference in activities among the different parts of the same plant recorded in this study may be associated with the presence of oils, wax, resins, fatty acids or pigments, which had been reported to be capable of blocking the active ingredients in the plant extract, thus, preventing the plant extract from accessing the bacterial cell wall [22].

The leaf extract of *M. indica* showed higher antibacterial activity to gram negative bacteria compared to gram positive bacteria. Two important pathogens such as, *E. coli* and *S. typhi* are highly susceptible to *M. indica* leaf extract.

The activities of *C. papaya* fruit extract and *C. papaya* seed extract were proportional. Both extracts of *C. papaya* were more active against gram positive bacteria than gram negative bacteria while that of the both extracts were next to the most sensitivity with the gram negative bacteria especially *S. typhi*.

The variation of antibacterial activities of the different extracts can be rationalized in terms of the polarity of the solvents used, polarity of the compounds being extracted from each solvent and in addition to their extrinsic bioactivity and by their ability to dissolve or diffuse in the media used in the assay [23]. Furthermore, it is seen that the diameter of the zones of inhibition of the leaf extracts increases as their concentrations increases, with exception of *P. guajava* bark extract which showed no zones of inhibition at all.

The extracts were active against both gram negative and gram positive bacteria tested and indicated a broad spectrum of activity. Demonstration of antibacterial activity against the test isolates is an indication that there is possibility of sourcing alternative antibacterial substances from these plants for the development of newer antibacterial agents. Further investigations of its activity against a wider range of bacteria, identification and purification of its chemical constituents, and toxicological investigations of the plant extracts should be carried out with a view to developing novel drugs for human consumption.

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