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Antibacterial and phytochemical screening of ethanol extracts of *Manilkara zapota* leaves and bark

Md. Rafikul Islam, Mst. Shahnaj Parvin, Mst. Rikta Banu, Nusrat Jahan, Nandita Das and Md. Ekramul Islam*

Dept. of Pharmacy, University of Rajshahi, Rajshah-6205, Bangladesh

* Corresponding author: Md. Ekramul Islam; e-mail: ekram@ru.ac.bd

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ABSTRACT

The ethanol extracts of leaves and stem bark of *Manilkara zapota* (L.) were examined for their antimicrobial properties and phytochemical screening. The agar gel diffusion method was used to determine the inhibitory effects of both the extracts against five gram positive and eight gram negative bacteria. The bark extract (400 µg/disc) showed significant antibacterial activity against all tested bacteria with the average value of 7-13.5 mm zone of inhibition. The leaves extract had lower actions at the same concentration and no activity was observed against one (*Staphylococcus aureus*) among the thirteen tested bacteria. Kanamycin (30 µg/disc) was used as standard antimicrobial drug whose zone of inhibition ranges from 16.5 to 25 mm. Preliminary phytochemical screening of the extracts revealed the presence of different types of phytochemical constituents including alkaloids, flavonoids, saponins and tannins.

Keywords: Antimicrobial, Phytochemical analysis, *Manilkara zapota,* Ethanol extract

INTRODUCTION

Infectious diseases caused by bacteria, viruses, fungi and other parasites are major causes of death, disability, and social and economic disruption for millions of people [1-3]. Approximately 15 million people die each year due to infectious diseases – nearly all live in developing countries. South Asia and sub-Saharan Africa do infectious diseases account for more than half of all deaths. In the developing world – where poverty and inadequate access to healthcare remain oppressive reminders of human frailty – infectious disease continues to be an omnipresent threat to life and livelihood. Moreover, the incidence of many diseases widely presumed to be under control such as cholera, dengue, yellow fever, and tuberculosis (TB) has increased in many areas or spread to new regions or populations throughout the world [4-8]. Because of widespread use and misuse of antimicrobial drugs, their effectiveness in treating common bacterial infections is diminishing, resulting in prolonged illnesses, higher mortality rates, and higher health-care costs [9-15]. However, emerging, re-emerging, and novel infections illustrate that no nation can be complacent regarding human vulnerability to microorganisms in the environment.

Nature has been a source of medical treatments for thousands of years and today plant based systems continue to play an essential role in the primary health care of 80% of the world's population []16, 17]. Knowledge on medicinal plants sometimes means the only therapeutic resource of some communities and ethnic group [18]. A large number of populations in the developing countries still depend on traditional medicine for their health care needs due to its affordability, accessibility and cultural importance. So, research has gained momentum to establish a scientific basis of folkloric use of medicinal plants and for the development of new agents from plant extracts effective against infections currently difficult to treat. Among the several examples, *Plantago major* Linn. and *P. asiatica* Linn. (Plantaginaceae) are commonly used plants as folk medicine in Taiwan for the treatment of infectious diseases, exhibited lymphocyte proliferation and secretion of interferon-gamma (IFN-γ) at low concentrations. Both lymphocyte proliferation activity and induced secretion of IFN-γ are indicators of cell-mediated immune response modulation [19]. Furthermore, Schelz and co-workers proved the antispasmodic activity of peppermint oil and its main

constituent, menthol, which means that menthol-containing substances are potential agents that could eliminate the resistance plasmids of bacteria. The main point of this menthol-induced plasmid elimination is a special mechanism of action and confirmed the relevance of peppermint oil and menthol as adjuvant antimicrobial agents [20]. In the continuation of this strategy of new drug, we have studied *Manilkara zapota* for its antibacterial properties and phytochemical composition.

Manilkara zapota (L.) commonly known as Sapodilla or Sofeda belongs to the family Sapotaceae. The sapodilla is a fairly slow growing, long-lived small to medium evergreen tree. It is strong and wind-resistant, rich in white, gummy latex. Its leaves are highly ornamental, evergreen, and glossy; flowers are small and bell-like and delicious fruits may be nearly round, oblate, oval. It is found all over the different parts of Bangladesh, Pakistan and India [21, 22]. It has been used in the indigenous system of medicine for the treatment of various ailments. Decoction of the bark used for diarrhea and fever. An infusion of the young fruits and the flowers is drunk to relieve pulmonary complaints and fever. Leaf decoction used for fever, hemorrhage, wounds and ulcers. The crushed seeds have a diuretic action and are claimed to expel bladder and kidney stones and effective in rheumatism. For neuralgia, leaves with tallow or oil, applied as compress to the temples [23]. Previously we reported that ethanolic extract of stem bark of the *M. zapota* possess antioxidant activity [24]. Here, the study was aimed at screening the leaves and stem of *M. zapota* for their antibacterial activities and evaluating their potential uses as safer drugs.

MATERIALS AND METHODS

Preparation of Extract

The matured leaves and bark were collected in the month of July 2009 from Rajshahi, Bangladesh. The leaves and bark were dried under shade and pulverized in a mechanical grinder. The powders were extracted with ethanol. The extracted juices were filtered by using a clean cloth and then filter paper. The extracts were concentrated first in Rotary vacuum evaporator and then on water bath. The extracted residues were weighed and percent yield of leaves and bark of *M. zapota* were 7.12 and 8.75 % w/w respectively. The extracts were then kept in refrigerator for antimicrobial properties and phytochemical investigations.

Antibacterial Assay

The disc diffusion method was used to test antimicrobial activity against thirteen bacteria [25]. Solutions of known concentration (400 mg/ml) of the test samples were made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with known amounts of the test substances using micropipette. Discs containing the test material were placed on nutrient agar medium uniformly seeded with the pathogenic test microorganisms. Standard antibiotic discs (Kanamycin 30 µg/disc) and blank discs (impregnated with solvents) were used as a positive and negative control. These plates were then kept at low temperature (4°C) for 1 hour to allow diffusion. There was a gradual change in concentration in the media surrounding discs. The plates were then incubated at 37°C for 12 hour to allow growth of the organisms. The test materials having antibacterial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. The antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter. The experiment was carried out three times and the mean of the reading is required. The antibacterial activity of ethanol extract of leaves and bark of *M. zapota* were determined at a concentration of 400 µg/disc.

Phytochemical Analysis

Test for Alkaloids

The presence of alkaloids is determined using the mayers and wagners test as described by the Harbone (1988) [26]. In this process, two gram of each portion of the powered sample are put into a conical flask and 20 ml of dilute sulphuric acid in ethanol are added into it and then heated in water bath to boil for 5 min. The mixture is filtered and the filtrates are separated treated with 2 drops of Mayers and Wagners reagents in test-tubes. Development of an orange coloration indicated positive result.

Test for Saponins

The Froth test and Emulsion test as described by Harbone (1973) are used to determine the presence of saponins [27]. In this process, twenty ml of water is added to 0.25 gm of the powdered sample in 100 ml beaker, boiled and filtered for the test.

Froth test: 5 ml of the filtrate is diluted with 20 ml of water and shaken vigorously. A stable froth (foam) up on standing indicates the presence of saponins.

Emulsion test: 2 drops of olive oil is added to the frothing solution and shaken vigorously the formation of emulsion indicates the presences of saponins.

Test for Tannins

The presence of tannins is carried out using the Harbone (1973) method [27]. 1gm of the powdered sample is boiled with 50 ml of water filtered and the filtrate used to carryout the ferric chloride test few drops of ferric chloride is added to 3 ml of the filtrate in a test tube. A greenish black precipitate indicates the presence of tannins.

Test for Flavonoids

The presence of flavonoids in the samples is determined using the Harbone (1973), Sofowora (1993) methods [28]. 10ml Ethyl acetate is added to 0.2gm of the powdered sample and heated in a water

bath for 5 min. The mixture is cooled filtered and the filtrates used for the test.

Ammonium test- About 4 ml filtrate is shaken with 1 ml of dilute ammonia solution. The layer is allowed to separate and the yellow colour in the ammoniacal layer indicates the presence of flavonoids.

Aluminum chloride solution test: 1 ml of 1% aluminum chloride solution is added to 4 ml of the filtrate and shaken. A yellow coloration indicates the presence of flavonoids.

RESULTS AND DISCUSSION

Antibacterial assay

The ethanol extracts (400 µg/disc) of the *M. zapota* leaves and bark were screened against thirteen bacteria to check antimicrobial activities by disc diffusion method. The extracts showed various level of antimicrobial activity against all bacterial strains with the different zone of inhibition (Table-1). Among tested bacteria, *Bacillus cereus, Escherichia coli, Shigella dysenteriae* are more sensitive to both extracts. Generally, bark extract of the *M. zapota* had higher antimicrobial activity than the extract from leaves against all tested microorganisms except *Streptococcus agalactiae* and *Proteus.* Leaves extract showed maximum activity against *Proteus* whereas highest zone of inhibition was found against *Shigella dysenteriae* for bark extracts. No zone of inhibition was found for negative control disc. The result was shown in the Table 1.

Table 1. *In vitro* antibacterial and antifungal activity of leaves, bark and standard kanamycin disc

Test organisms	Diameter of zone of inhibition (mm)		
	Leaves extract (400µg/ disc)	Bark extract (400µg/ disc)	Kanamycin (30 µg/disc)
Gram positive bacteria			
Staphylococcus aureus	-	9	25
Streptococcus agalactiae	8	7	21
Bacillus cereus	8.5	9	21
Bacillus megaterium	7	13	23
Bacillus subtilis	7	8.5	23
Gram negative bacteria			
Pseudomonas aeruginosa	7.5	11	16.5
Proteus vulgaris	9	7.5	20
Escherichia coli	8.5	13	24
Shigella flexneri	7	13	21
Shigella dysenteriae	8	13.5	19
Shigella sonnei	7	12.5	19
Shigella boydii	7	13	18.5
Shigella shigae	6.5	12	20

Phytochemical analysis

The preliminary phytochemical screening of different extracts was done to ascertain the presence of bioactive components. The result was shown in Table-2. Both the extracts in our study showed positive result to all components tested and indicated the presence of alkaloids flavonoids, tannins, and saponins in the leaves and bark extracts of *M. zapota*.

Table 2. Phytochemical screening of extracts of the *M. zapota* leaves and bark

Sample	Alkaloids	Flavonoids	Saponins	Tannins
Leave extract	+	+	+	+
Bark extract	+	+	+	+

Here, positive sign [+] indicates the presence of component

Plants produce a huge variety of secondary compounds as natural protection against microbial and insect attack. Some of these compounds are toxic to animals, but others may not be toxic. Indeed, many of these compounds have been used in the form of whole plants or plant extracts for food or medical applications in human because plants are the natural reservoir of many antimicrobial, antifungal, insecticidal, anticancer, analgesics, anti-diarrheal agents, as well as various therapeutic activities [29, 30]. Acceptance of medicines from such plant origin as an alternative form of healthcare is increasing because they are serving as promising sources of novel antibiotic prototypes [31, 32]. Some of the phytochemical compounds e.g. alkaloids, saponins, tannins, flavonoids, terpenoids and glycosides have variously been reported to have antimicrobial activity [33, 34].

The present study revealed that the ethanol extracts leaves and bark of *M. zapota* have got antimicrobial activities might be due to the presence of some sorts of bioactive or inhibitory compounds or factors in the extracts or synergism by the existence of some compounds or factors in the extract. Since a variety of constituents are present in the extract studied; so, further extensive investigations are necessary to find out the active antimicrobial principles present in this plant parts that may lead to the development of noble antibiotic in future.

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