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Antimicrobial Activity of Ethanol and Chloroform Extracts of Leaves and Stem Bark of *Manilkara zapota* L.

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ABSTRACT

In vitro antimicrobial activities of crude chloroform and ethanolic extracts of various plant parts of *Manilkara zapota* L. was investigated. The extracts exhibited antimicrobial activities with zones of inhibition ranging from 6mm to 16mm. All the extracts exhibited appreciable activity against all the clinically important bacterial and fungal species investigated. In *Manilkara zapota* L. maximum zone of inhibition (16mm) was observed in ethanolic extract of leaves against *Streptomyces grisveus* and minimum in ethanolic extract of stem bark (6mm) against *Fusarium oxysporum*. The antimicrobial activity of the extract was compared with the standard drugs. The ability of the crude extracts of *Manilkara zapota* L. plant parts to inhibit the growth of various bacteria and fungi showed its broad-spectrum antimicrobial potential, which may be employed in the management of microbial infections. Hence this study offers a base of using *Manilkara zapota* L. as herbal alternative for the synthesis of antimicrobial agent.

***Key words*:** Antimicrobial, Antifungal, Antibacterial, Zone of inhibition, *Manilkara zapota* L.

Sapodilla is a deciduous tree with dense and round canopy formed by profuse branching. Initially the growth of plant reaches upto 20-30 m in height. The plant exudes milky latex known as "chicle" [1]. Traditionally, the leaves of *Manilkara. zapota* L. have been used against cold, cough and diarrhoea [2] and have good potential for analgesic, antihyperglycemic and hypocholesterolemic activities [3]. Fruit are being used to treat diarrhoea and pulmonary diseases. While, crushed seeds are used to treat stones of bladder and kidney as well as rheumatism, leaf decoction is used to cure fever, haemorrhage, wounds and ulcers. The bark of this plant is also routinely used for the treatment of gastrointestinal disorder, fever, pain and inflammation [4]. Antimicrobial therapy was developed in the first half of the last century. The development of resistance in many bacterial and fungal strains however constitutes one of the most serious problems in the control of most infectious diseases. The screening of plant products for antimicrobial activity has shown that the higher plants represent a potential source of novel antibiotic prototypes [5]. There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. Even after antimicrobial potency of certain medicinal plants has been reported still there is an urgent need to discover new antimicrobial compounds from plants with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases [6].

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MATERIALS AND METHODS

Fresh and disease-free plant parts of *Manilkara zapota* L. (leaves and stem bark) was collected from the Jhunjhunu district of Rajasthan. The collected plants were identified and authenticated by the Department of Botany, University of Rajasthan, Jaipur.

Determination of antimicrobial activity

Plants parts were shade dried and extracted with respective solvents of Chloroform and Ethanol.

Preparation of extract

The crude extract was obtained by macerating 30 g of dried plant powder in 95% in respective solvents and kept on a rotary shaker for 24 hrs. The extract was filtered, centrifuged at 5000g for 15 mins and was dried under reduced pressure. The extract was stored at 4ºC in airtight bottles.

Culture and maintenance of clinical isolates

Pure cultures of *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Streptomyces griseus*, *Aspergillus niger*, *Fusarium oxysporium*, *Trichoderma reesei* and *Penicillium funiculosum* obtained from S.M.S. Medical

College, Jaipur, India was used as test organisms. Each culture was further maintained on the same medium after every 48 hrs of transferring. A fresh suspension of test organism in saline solution was prepared from a freshly grown agar slant before every antimicrobial assay.

Determination of antibacterial assay

In vitro antibacterial activity of the crude methanol extract was studied against gram positive and gram-negative bacterial strains by the agar well diffusion method [7]. Mueller Hinton agar no. 2 (Hi Media, India) was used as the bacteriological medium. The extracts were diluted in 100% Dimethylsulphoxide (DMSO) at the concentrations of 5 mg/mL. The Mueller Hinton agar was melted and cooled to 48-50°C and a standardized inoculum (1.5×108 CFU/mL, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile petri dishes to give a solid plate. Wells were prepared in the seeded agar plates. The test compound (100 µl) was introduced in the well (6 mm). The plates were incubated overnight at 37°C. The antimicrobial spectrum of the extract was determined for the bacterial species in terms of zone sizes around each well. The diameters of zone of inhibition produced by the agent were compared with those produced by the commercial control antibiotics, streptomycin. For each bacterial strain controls were maintained where pure solvents were used instead of the extract. The control zones were subtracted from the test zones and the resulting zone diameter was measured with antibiotic zone reader to nearest mm. The experiment was performed three times to minimize the error and the mean values are presented.

Determination of antifungal assay

Antifungal activity of the experimental plant was investigated by agar well diffusion method [8]. The yeasts and saprophytic fungi were subcultured onto Sabouraud's dextrose agar, SDA (Merck, Germany) and respectively incubated at 37°C for 24 hrs and 25°C for 2-5 days. Suspensions of fungal spores were prepared in sterile PBS and adjusted to a concentration of 106 cells/ml. Dipping a sterile swab into the fungal suspension and rolled on the surface of the agar medium. The plates were dried at room temperature for 15 min. Wells of 10 mm in diameter and about 7 mm apart were punctured in the culture media using sterile glass tube. 0.1 ml of several dilutions of fresh extracts was administered to fullness for each well. Plates were incubated at 37°C. After incubation of 24 hrs bioactivities were determined by measuring the diameter of inhibition zone (in mm). All experiments were made in triplicate and means were calculated.

RESULTS AND DISCUSSION

In *Manilkara zapota* L., the antimicrobial activity of chloroform and ethanolic extracts of different plant parts were tested against four bacterial strains (*B. subtilis, E. coli, S. aureus* and *S. grisveus*) and four fungal strains (*A. niger, F. oxysporum, P. funiculosum* and *T. reesie*). The Zone of Inhibition (ZI) was measured by antibiotic zone reader. Ethanolic extract of stem bark and leaves shows ZI 12mm and 10mm respectively and chloroform extract of stem bark and leaves have no activity against *Bacillus subtilis.* In *Escherichia coli* ethanolic extract of stem bark and leaves shows ZI 14mm and 10mm respectively and chloroform extract of stem bark shows ZI 10mm and leaves have no activity. Ethanolic extract of leaves shows ZI 14mm and ethanolic extract of stem bark, chloroform extract of stem bark and leaves have no activity against *Staphylococcus aureus.* Ethanolic extract of leaves shows maximum ZI 16mm and ethanolic extract of stem bark, chloroform extract of stem bark and leaves have no activity against *Streptomyces grisveus* (Table 1).

Table 1 Antibacterial activities of various extracts of *Manilkara zapota* L. against selected bacterial strains

Bacterial strains	Various extracts	Leaves	Stem bark
		(Zone in mm)	
Bacillus subtilis	Ethanolic	ZI = 10 AI = 0.5	ZI = 12 AI = 0.6
	Chloroform	NA	NA
Escherichia coli	Ethanolic	ZI = 10 AI = 0.5	ZI = 14 AI = 0.7
	Chloroform	NA	ZI = 10 AI =0.5
Staphylococcus aureus	Ethanolic	ZI = 14 AI = 0.7	NA
	Chloroform	NA	NA
Streptomyces grisveus	Ethanolic	ZI = 16 AI = 0.8	NA
	Chloroform	NA	NA

Zone of Inhibition = ZI (in mm), Activity index = AI and No Activity = NA, Activity index (AI) = Zone of Inhibition of the Sample/ Zone of Inhibition of the Standard

Ethanolic and chloroform extract of leaves and stem bark have no activity against *Aspergillus niger.* In *Fusarium oxysporum* maximum ZI was in ethanolic extract of stem bark 6mm and ethanolic extract of leaves, chloroform extract of stem bark and leaves have no activity. In the case of *Penicillium funiculosum* ethanolic extract of leaves and stem bark shows ZI 14mm and 8mm respectively and chloroform extract of leaves and stem bark have no activity against. Ethanolic extract of leaves and stem bark shows ZI 8mm and 8mm respectively and chloroform extract of leaves and stem bark have no activity against *Trichoderma reesie.* (Table 2). In *Manilkara zapota* L. ethanolic extract of stem bark showed maximum activity against *Escherichia coli* and ethanolic extract of leaves shows maximum activity against *Streptomyces grisveus.* In the case of *Penicillium funiculosum* ethanolic extract of leaves and stem bark showed maximum activity. An antimicrobial is an agent that kills microorganisms or inhibits their growth. Antimicrobial

medicines can be grouped according to the microorganisms they act primarily against. Antibacterial, commonly known as antibiotics, are used against bacteria and antifungal are used against fungi. They can also be classed according to their function [9]. Plant products still remain the principle source of pharmaceutical agents used in traditional medicine [10]. Phytochemicals are the compounds naturally occurring in plants. Some are responsible for colour and other organoleptic properties, such as the deep purple of blueberries and the smell of garlic. Plant produces various compounds which are responsible for protection against pathogens. Some plants may contain compounds of potential medical use [11-12]. Medicinal plants are very important in our daily life as these are used for the treatment of many diseases. It has been used from ancient time and now days they are used as food supplement, nutraceuticals, in modern medicines. In the present investigation initial screenings of the experimental plant for possible antimicrobial activities was done using crude methanolic extracts. Nearly all of the identified components from plants that are active against microorganisms are aromatic or saturated organic compounds and most often obtained through ethanol or methanol extractive. In the present study *Manilkara zapota* L. showed antimicrobial potent activity against fungal strains as compared to bacterial strain.

Table 2 Antifungal activities of various extracts of *Manilkara zapota* L. against selected fungal strains

fungal strains	Various extracts	Leaves		Stem bark	
		(Zone in mm)			
Aspergillus niger	Ethanolic	NA		NA	
	Chloroform	NA		NA	
Fusarium oxysporum	Ethanolic	NA		ZI = 6	AI = 0.27
	Chloroform	NA		NA	
Penicillium funiculosum	Ethanolic	ZI = 14	AI = 0.63	ZI = 8	I = 0.36
	Chloroform	NA		NA	
Trichoderma reesei	Ethanolic	ZI = 8	AI = 0.36	ZI = 8	AI = 0.36
	Chloroform	NA		NA	

Zone of Inhibition = ZI (in mm), Activity index = AI and No Activity = NA, Activity index (AI) = Zone of Inhibition of the Sample/ Zone of Inhibition of the Standard

CONCLUSION

The present study indicates that *Manilkara zapota* L. extracts have broad inhibitory activities to pathogenic microorganism and to act as potential antimicrobial agent from natural sources. In general, commercial antibiotic and antifungal drugs causes side effects such as liver, kidney and gastrointestinal tract toxicity. Severe hepatotoxicity had also been reported in patients undergoing antifungal drug therapy. However, herbal remedies often do not produce any side effects. Therefore, alternative medicine become popular remedy to various types of ailments. In conclusion, *Manilkara zapota* L. extracts have revealed significant antimicrobial activities against selected strains for experiment.

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