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Response Of Various Extracts Of Manilkarazapota (L) Seeds Against Periodontitis Triggering Microbiota

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Abstract

Oralmicrobiota to predominate periodontitis andadjustment of human pathogen shifted microbiota by plants intended present study to evaluate the response of various extracts of Manilkara zapota (L) seeds against periodontitis triggering pathogens. Present study involvedpreparation of methanol, acetone, petroleum ether, ethanol, and ethyl acetate extracts ofManilkara zapota (L) seeds stems using Soxhlet extractor.The prepared extracts were further evaluated for their potential to inhibit the periodontitis triggering pathogens and subjected to phytochemical screening. The phytochemical screening of different extracts showed presence of alkaloid, flavonoids, glycosides, carbohydrates, and proteins. Among all extracts, the methanol extract of Manilkara zapota seeds showed best antimicrobial profile against strains of Vibrio cholera, Salmonella parthyphi A, andShigellaflexnari. Present study concludes methanolic extract of Manilkara zapota seeds to possess high inhibitory potential against periodontitis triggering pathogens. The high inhibitory potential of methanolic and acetone extract of Manilkara zapota seeds extract could be attributed to the presence of alkaloidal and flavonoidal constituents.

Key Words: Periodontitis,antimicrobials, phytochemical screening, Manilkara zapota, bacteria

1. Introduction

Human body is known to possesshuman pathogenic and non-pathogenic bacteria in a symbiotic relationship, but a little disturbance could manifest invarious infections [1,2]. Periodontitis (PD) is a chronic inflammation disease of supporting tissues of teeth. PD is triggered by complex interaction between different micro-organisms and immune response of host [3-5].Fact suggest Staphylococcus aureus, Klebsiella pneumoniae, Staphylococcus aureus, Bacillus subtilis, Bacillus thuringiensis, Vibrio cholera, Escherichia coli, and Shigellaflexneri to trigger periodontitis. The extensive and inappropriate use of antibiotics may lead to emergence of antibiotic-resistance. Development of microbe's resistance and patient tolerance reduces the efficacy of commercial antibiotics and increases the demand of alternative antibiotics [6-10]. Plants are known to possess various bio-constituents, such as alkaloids, phenols, polyphenols, terpenoids, flavonoids, flavonols, and glycosides; that are responsible for several biological and antimicrobial activities [11-35].Evidence suggests plants to possess strong antimicrobial potentialagainst micro-organism (attributed to their defence mechanism) [36-40].Manilkara zapota (L.) plant known as the sapodilla belongs to Sapotaceae family, containing bioactive polyphenolic compounds ca be a good source for antimicrobial compounds.M. zapotais known to exhibit antioxidant, hypoglycemic, anti-inflammatory, anti-bacterial, and anti-tumoractivities[41-46]. Manilkara zapota (L) plant is rich sources of saponin, tannin, sugars, proteins, ascorbic acid, phenolics, carotenoids, alkaloids, glycosides, carboxylic acids [47-49].Emergence of bacterial resistance to conventional antibiotics creates the need for the search for antibacterial agents from different sources [50-52]. Hence the present study was done to explore the antibacterial potential of the Manilkara zapota (L) seeds against the pathogenic bacteria that triggers periodontitis.

2. Material and methods

The Manilkara zapota fruits were collected from the premise of Lunas in Kulim district of Kedah state of Malaysia, in the month of October. The flesh of fruits was removed to obtain seeds. The outer layer of seeds was removed, and kernels so obtained were dried in natural sunlight. The chemical and reagents used in the study like streptomycin, methanol, ethanol, acetone, ethyl acetate, and petroleum ether, sulphuric acid and sodium hydroxide were of synthetic and laboratory grade and were procured from Merck, Germany, Abbott Lab. USA. Muller Hinton Agar nutrient media was purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. The chemicals used in phytochemical test such as hydrochloric acid, Fehling solution, lead acetate, iron (III) chloride and sodium chloride were obtained from R & M Marketing UK and Bendason Laboratory Chemicals.

2.1. Preparation of Manilkara zapota seeds extracts

The Manilkara zapota seeds were separated from fruits. The seeds so obtained were subjected to removal of kernel, cleaned with distilled water and further dried in natural sunlight. The dried seeds were further crushed to coarse powder using mortar and pestle. The obtained powder was further subjected to different solvent extraction using Soxhlet apparatus [53-64]. The Manilkara zapota dried seeds powder was subjected to successive extraction with petroleum ether, ethyl acetate, acetone, ethanol, and methanol in increasing order of polarity. After successive Soxhlet extraction with different solvents, the different solvent extracts were subjected to purification. The solvent was removed using rotavapor and evaporation method to get the pure extracts [65-71].

2.2. Responseof Manilkara zapota Extracts Against PeriodontitisTriggering Pathogens

2.2.1. Preparation of bacterial culture

Eight bacterial strains such as: Staphylococcus aureus, Klebsiella pneumoniae, Staphylococcus aureus, Bacillus subtilis, Bacillus thuringiensis, Vibrio cholera, Escherichia coli, and Shigellaflexneriwere used for the antimicrobial experiment.The prepared stock culture of microorganism weremaintained at 4°C.Subcultures were prepared by transferring loopful of microorganisms colonies from stock cultures into the nutrient broth and incubated for 24 hours at 37˚C in the incubator. The broth turbidity indicated the microbial growth[72-80].

2.2.2. Well Diffusion Method

The inhibitory potential of the different prepared extracts of Manilkara zapota seedsagainst periodontitis triggering pathogens was determined using well diffusion method-based zone of inhibition. The experimental protocol was followed as per the standard references with slight modifications. Briefly, 20 µl of nutrient broth containing broth organism was poured into Muller Hinton agar plate, that was spread uniformly using L-shape rod. The wells were made on the agar medium with cork borer of 5 mm in diameter which was previously sterilized using autoclave at 121ºC for one hour. Each 50 µl of plant extracts were pipetted separately into the cup made on the agar plate. In the agar plate a few wells for extracts, standard and control. These plates contained the antibiotic streptomycin (standard) and tween 80 (control) solution for the purpose of comparison with the plant extracts. All the plates were incubated for 24 hours at 37ºC. The diameter of zone of inhibition around wells was measured in milliliters (mm) in triplicate and average values were calculated [81-85].

2.3. Preliminary Phytochemical screening of Manilkara zapota Extracts

The petroleum ether, ethyl acetate, acetone, ethanol, and methanol extracts of Manilkara zapota seeds were subjected to preliminary phytochemical screening for the detection of various plant constituents. The prepared extract were screened for the presence of alkaloids, carbohydrates, flavonoids, glycosides, proteins, tannins, and phenols as per the procedure given in standard references [86-91].

3. Results

3.1. Response of Manilkara zapota Extracts Against Periodontitis Triggering Pathogens

In present study, the prepared extracts of Manilkara zapota seeds were evaluated for their inhibitory potential against periodontitis triggering bacteria such as S. aureus, K. pneumoniae, S. aureus, B. subtilis, B. thuringiensis, V. cholera, E. coli, and Shigellaflexneriusing Agar well diffusion for measurement of zone of inhibition. The prepared extracts of Manilkara zapota seedswere evaluated for their antimicrobial potential against various bacterial strains using well diffusion method. The results so obtained are given in table 1 to 5.

Table 1: Zone of inhibition of the methanolic extract of Manilkara zapota

Microorganism	**Component**	**Zone of inhibition (mm)**			
		Reading 1	**Reading 2**	**Reading 3**	**Average value**
Escherichia coli	10%	-	-	-	
	20%	-	-	-	
	Streptomycin	22	20	22	21.3
	Control	-	-	-	
Klebsiella pneumoniae	10%	-	-	-	
	20%	-	-	-	
	Streptomycin	18	17	20	18.3
	Control	-	-	-	-
Staphylococcus aureus	10%	-	-	-	-
	20%	-	-	-	-
	Streptomycin	19	21	18	19.3
	Control	-	-	-	
Vibrio cholera	10%	21	23	21	21.7

	20%	26	24	23	24.3
	Streptomycin	26	26	25	25.6
	Control	-	-	-	-
Salmonella paratyphi A	10%	26	26	22	24.6
	20%	23	27	26	25.3
	Streptomycin	26	27	26	26.3
	Control	-	-	-	-
Shigellaflexneri	10%	22	25	26	24.3
	20%	25	27	27	26.3
	Streptomycin	26	27	27	26.6
	Control	-	-	-	-

Table 2: Zone of inhibition of the ethanolic extract of Manilkara zapota

Microorganism	**Component**	**Zone of inhibition (mm)**			
		Reading 1	**Reading 2**	**Reading 3**	**Average value**
Escherichia coli	10%	-	-	-	-
	20%	-	-	-	-
	Streptomycin	17	19	15	17
	Control	-	-	-	-
Bacillus thuringiensis	10%	-	-	-	-
	20%	-	-	-	-
	Streptomycin	23	20	22	21.6
	Control	-	-	-	-
Staphylococcus aureus	10%	-	-	-	-
	20%	-	-	-	-
	Streptomycin	26	26	27	26.3
	Control	-	-	-	-
Bacilllus subtilis	10%	-	-	-	-
	20%	-	-	-	-
	Streptomycin	19	19	22	20
	Control	-	-	-	-

Table 3: Zone of inhibition of the ethyl acetate extract of Manilkara zapota

Microorganism	Component	Zone of inhibition (mm)			
		Reading 1	Reading 2	Reading 3	Average value
Escherichia coli	10%	-	-	-	-
	20%	-	-	-	-
	Streptomycin	18	18	19	18.3
	Control	-	-	-	-
Bacilllus subtilis	10%	-	-	-	-
	20%	-	-	-	-
	Streptomycin	22	24	21	22.3
	Control	-	-	-	-

Table 4: Zone of inhibition of the petroleum ether extract of Manilkara zapota

Microorganism	Component	Zone of inhibition (mm)			
		Reading 1	Reading 2	Reading 3	Average value
Escherichia coli	10%	-	-	-	-
	20%	-	-	-	-
	Streptomycin	22	25	25	24
	Control	-	-	-	-
Bacilllus subtilis	10%	-	-	-	-
	20%	-	-	-	-
	Streptomycin	17	19	21	
	Control	-	-	-	-

Table 5: Zone of inhibition of the acetone extract of Manilkara zapota

Microorganism	Component	Zone of inhibition (mm)			
		Reading 1	Reading 2	Reading 3	Average value
Escherichia coli	10%	-	-	-	-
	20%	-	-	-	-
	Streptomycin	23	21	24	22.6

	Control	-	-	-	-
Bacilllus subtilis	10%	-	-	-	-
	20%	-	-	-	-
	Streptomycin	17	19	20	18.6
	Control	-	-	-	-
Vibrio cholera	10%	20	21	21	20.6
	20%	23	22	22	22.3
	Streptomycin	26	26	25	25.6
	Control	-	-	-	-

'+' indicates no zone of inhibition

3.2. Preliminary Phytochemical screening of Manilkara zapota Extracts

The extracts of Manilkara zapota (L) seeds were subjected to qualitative testing as per the procedure given in standard references. The list of compounds identified in methanol, acetone, petroleum ether, ethyl acetate and ethanol extract of Manilkara zapota (L) seeds are given in table 6.

Table 6: Phytoconstituents of the various extracts of Manilkara zapota seeds

S. No.	Phytoconstituents	Type of extract				
		Methanolic	Acetone	Petroleum ether	Ethyl acetate	Ethanol
1	Alkaloids	+	-	+	+	-
2	Carbohydrates	+	-	-	-	+
3	Flavonoids	+	-	-	-	-
4	Glycosides	+	-	-	-	+
5	Proteins	-	+	+	+	-
6	Tannins and phenolic compounds	+	+	+	+	-
7	Sterols	-	+	+	-	-

'+' indicates present

'-' indicates absent

4. Discussion

Evidence reports Staphylococcus aureus, Klebsiella pneumoniae, Staphylococcus aureus, Bacillus subtilis, Bacillus thuringiensis, Vibrio cholera, Escherichia coli, and Shigellaflexneri to trigger periodontitis. The growing incidences of microbial resistance towards conventional antibiotics raises the demand for evaluation of alternative antimicrobials [2-5,92-97]. Reports suggestsuse of Manilkara zapotain the treatment of various diseases and to possess strong antimicrobial potential. As per the literature available over different parts of Manilkara zapota plant and very less literature was available over antimicrobial potential of Malaysian Manilkara zapota plant seeds. Hence, investigators of present study planned to evaluate the in-vitro inhibitionpotential of Manilkara zapota seeds extracts against periodontitis triggering pathogens (Staphylococcus aureus, Klebsiella pneumoniae, Staphylococcus aureus, Bacillus subtilis, Bacillus thuringiensis, Vibrio cholera, Escherichia coli, and Shigellaflexneriusing well diffusion method.The extracts of Manilkara zapotaseedswere prepared by successive extraction with different solvents such as petroleum ether, ethyl acetate, acetone, ethanol, and methanol in increasing order of polarity. Different solvent extracts of Manilkara zapota seeds were investigated for their anti-microbial activity (using well diffusion method) and phytochemical screening. The methanolic extract of Manilkara zapota seeds was investigated for its zone of inhibition against E. Coli,Klebsiella pneumoniae, Staphylococcus aureus, Vibrio cholera, Salmonella paratyphi A, and Shigellaflexneri. The methanolic extract showed good inhibitory effect overgrowth of Vibrio cholera, Salmonella parathyphi A, and Shigellaflexneri. WhereasKlebsiella pneumoniae and Staphylococcus aureus were not found susceptible to methanolic extract. The ethanolic extract of Manilkara zapota seeds was investigated for its antibacterial potential against E. coli, Bacillus thuringiensis, Staphylococcus aureus and Bacillus subtilis. The ethanolic extract was found to remain ineffective against all tested bacterial strains such that no zone of inhibition was detected. The ethyl acetate extract and petroleum ether extracts of Manilkara zapota seeds were investigated for their antibacterial potential against E. coli and Bacillus subtilis. The two extracts were found to be ineffective against all tested bacterial strains such that no zone of inhibition was detected. The acetone extract of Manilkara zapota seeds was investigated for their antibacterial potential against Vibrio cholera, E. coli, and Bacillus subtilis. The acetone extract showed good antibacterial potential against all tested Vibrio cholerae. The different solvent extracts of Manilkara zapota seeds were also subjected to phytochemical testing to identify the nature of compound present in the extracts. The methanolic extract was found to possess alkaloids, carbohydrates, glycosides and proteins. Acetone extract showed presence of alkaloids and flavonoids. Ethyl acetate extract and ethanol extracts showed presence of protein.

5. Conclusion

After conducting the present study and reviewing the inhibitory potential of different solvent extracts of Manilkara zapota seeds against periodontitis triggering pathogens, it is here by concluded that methanolicextract of Malaysian Manilkara zapota seeds possess good antimicrobial activity towards bacterial strains of Vibrio cholera, Salmonella paratyphi A, and Shigellaflexneri. Among methanol and acetone extracts, the antimicrobial potential of methanolicextract is high against periodontitis triggering pathogens. The high antimicrobial potential of methanolic extract of Manilkara zapota seeds extract must be due to presence of alkaloidal and flavonoidal constituents.

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