



A RESEARCH ON MANILKARA ZAPOTA SEEDS EXTRACT ON ANTI-MICROBIAL ACTIVITY AGAINST CLINICAL PATHOGEN

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ABSTRACT

Manilkara zapota is a medicinal herb belongs to the family Sapotaceae. The on parts of plants like Seeds are extracted by using the solvent methanol. The methanol extracts were screened for the antimicrobial activity. They showed greater inhibitory effect against both gram positive and gram negative organisms. The organisms used were such as Escherichia coli, Salmonella typhi, Escherichia coli, proteus mirabilis. Based on the present investigation results it is concluded that the methanolic extracts of Manilkara zapota has great potential as antimicrobial agent against different microorganisms and they can be used in the treatment of infectious diseases caused by the resistant microorganisms.

KEYWORDS: Anti-microbial Activity, Manilkara zapota, clinical Pathogen, Disc Diffusion Method.

INTRODUCTION

India is widely known as the botanical garden of the world since it is the largest producer of medicinal herbs.^[1] Medicinal plants continue to be an important Pharmacological and therapeutic aid for improving the ailments of humankind. The search for eternal health and longevity for remedies to relive pain and discomfort drove early man to explore his immediate natural surroundings to the use of various plants, animal products and minerals etc for the development of a variety of therapeutic agents. Today, there is a renewed interest in traditional medicine and an increasing demand for more drugs from plants extract sources. 80% of the population of developing countries depend on traditional medicines, mostly natural plant products, for their primary health care needs as estimated by WHO.^[2] Because of the growing appreciation of natural products the demand for medicinal plants has been growing all over the world. They have minimal toxicity, are cost effective and pharmacologically active, and provide an easy preparation for many human ailments as compared to the synthetic drugs which are a subject of adulteration and side effect.^[3] This renewal of interest in plant derived drugs is mainly due to the current widespread belief that "green medicine" is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects. The alarming increase in the rate of infection by antibiotic-resistant microorganisms has urged scientists to search for compounds which have potential antimicrobial activity.^[4] The effectiveness of plant extracts on microorganism has been studied

worldwide.^[5] Nature has blessed us with a wonderful fauna and flora, which has made our life beautiful. One of these miracles is a sapodilla fruit. The Sapotaceae family includes about 800 species and shrubs in around 65 genera.^[6] the scientific name Sapodilla is Manilkara zapota. belonging in to Family Sapotaceae. The fruit sapodilla is commonly known as chickoo, sapota, Nasebery. It possesses tremendous nutritional value. Sapodilla is regarded as natural energy booster as it contains sucrose, fructose.^[7] Present study we have selected a plant name Manilkara Zapota seeds belonging in family Sapotaceae. The manilkara zapota plant contain flavonoids, alkaloids. Sapodilla is a medium to large sized tree with dense and almost round canopy formed by profuse branching system. Initially the growth of these trees is slow but after many years, may reach up to 20-30 metres in height. All tree parts exude out milky latex known as "chicle".

Roots: Sapodilla roots show shallow-root system with a major portion of roots present within the top 75 cm of soil and about 66% of the moisture extracted from the soil is in the first 75 cm.

Leaves: The leaves are evergreen and spirally arranged (7-12×2-4cm in size), pinkish brown when young and turns light-dark green at maturity. Secondary veins make a wide angle with the midrib.

Flowers: Flowers are small, bisexual, bell-shaped (10 mm in diameter), borne singly or in clusters in the axils of leaves near the branch tips.

Fruit: The fruit of sapodilla is a brown coloured berry, nearly round and varies from 5-10 cm in width. The unripe fruit is hard and coarse whereas it becomes soft and juicy on maturity.

Seeds: Some Sapodilla fruits are seedless but normally they produce 3-12 seeds per fruit. They are hard and brown or black in colour with one white margin. The seeds contain some phytochemicals like sapotin, saponin, achras saponin- and the bittersapotonine. Hydrocyanic acid is also present in seeds, so should be removed before eating the fruit.^[8-9]

Reproductive biology: Sapodilla is an out-breeding, species and show self-incompatibility Honeybees collect nectar from the flowers and may contribute to the pollination. The flowers of self-incompatible variety undergo crosspollination by other sapodilla varieties so as to produce fruits. Flowering and fruiting occurs throughout the year; it takes about 4 months for fruits to mature. The seedlings start bearing fruits after 5-8 years, while the grafted varieties flower earlier (2-3 years from planting). Sapodilla is pollinated by insects.^[10-11]

Phytoconstituents: Of Sapodilla Various workers from time-to-time reported the presence of phytochemicals in leaf and seed extracts of Sapodilla.^[11] The Extraction of various phytochemicals from Sapodilla using three solvent like methanol, Acetone, and chloroform extracts. These workers observed the presence of Glycosides, saponins, Steroids in low polar chloroform extracts; and maximum number of phytoconstituents such as Phenol, tannins, flavonoids, alkaloids, steroids, glycosides and saponins were present in the medium polar acetone extract. steroids, phenols, glycosides and saponins in the high polar methanol extract.^[12] In sapodilla plants this

phytoconstituents produced in the seeds and other plant parts of sapodilla are responsible for their antioxidant activities and pharmaceutical application.

MATERIAL AND METHOD

Plant material: The fresh plant seeds of *Manilkara zapota* was collected locally from Chh.Samnahnagar. Maharashtra, authenticated as *Manilkara zapota* plant family- Sapotaceae. Collection, authentication, identification, processing and storage have been done according to standard procedure for the plant material.

Preparation of extract: The collected seed was dried under shade, segregated and further crushed to coarse powder by mechanical grinder and the powder was passed through sieve no 14. The dried powdered seeds of *Manilkara zapota* (250 gm) were first defatted with Petroleum Ether (60-80 °C) and later extracted with acetone & methanol by continuous hot extraction method in soxhlet apparatus. The extracts obtained were subjected to standardization and then utilized for evaluating *in-vitro* anti-microbial activity.

Bacterial strains: Five bacterial strains were employed for the test which include. *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *proteus mirabilis*, *Pseudomonas aeruginosa*.

Disc Diffusion Technique: The nutrient agar plates were prepared and the test bacterial strain was smeared on the Nutrient agar surface using sterile cotton swab. The antibiotic disc loaded with plant extract was placed on the surface of the Nutrient agar plates. Controls were maintained by loading dimethyl sulfoxide on disc. Then the plates were incubated at 37°C for 10 to 18 hours.

3. RESULTS

Phytochemical test of *Manilkara zapota* seed extracts.

(+) Present, (-) Absent

Table 1: Phytochemical test for MZ Extract.

Chemical test	Pet ether	Methanol	Acetone
A) Test for carbohydrate			
1) Molish test	+	-	+
2) Fehling's test	+	-	+
3) Benedict's test	+	-	+
B) Test for proteins			
1) Biuret test	+	+	+
2) Millions test	+	+	+
C) Test for amino acids			
1) Ninhydrin test	-	-	+
D) Test for steroid			
1) Salkowski test	+	+	+
2) Liebermann-Burchard reaction	+	+	+
E) Test for glycosides			
1) Bontrager's test	-	-	+
2) Foam test	-	+	+
F) Test for flavonoids			

1) Shinoda test	+	+	+
2) Lead acetate test	-	-	-
3) Sulphuric acid test	+	+	+
E) Test for tannins and phenolic compounds			
1) Lead acetate test	-	+	+
2) Ferric chloride test	-	+	+
3) Potassium dichromate	-	+	+
4) Dil. nitric acid test	—	+	+
F) Test for alkaloids			
1) Hager's test	+	-	+
2) Wagner's test	+	-	+
3) Dragendroff's test	+	-	-
4) Mayer's test	-	-	+

Media preparation for nutrient agar media

The bacterial cultures of gram positive and also gram negative bacteria were maintained on nutrient agar medium (agar 15-16 gm, beef extract 3 gm, peptone 5 and sodium chloride 5 gm in one liter distilled water). These micro-organisms were allowed to grow at 35°C-37°C at room temperature. A fresh inoculum of test microorganism in saline solution was prepared from a freshly grown agar slant before every anti-microbial assay by adjusting the concentration of micro-organism in the medium using spectronic-20 colorimeter (Bausch and Lomb) set at 630 nm, transmittance used bacteria was 40%.

Preparation of sterile disc

Sterile disc of size 5 mm diameter were obtained by using Whatman filter paper No.1 used for the present investigation. The extracts of medicinal plants were incorporated into the sterile disc. Each sterile disc was incorporated individually with 50,100,200,300 ppm. The discs were allowed to dry in laminar air flow. Then another dose of extract was applied. Assay of the

antibacterial activity of the medicinal plant extract were done by Disc diffusion technique.

Disc Diffusion Technique

The nutrient agar plates were prepared and the test bacterial strain was smeared on the Nutrient agar surface using sterile cotton swab. The antibiotic disc loaded with plant extract was placed on the surface of the Nutrient agar plates. Controls were maintained by loading dimethyl sulfoxide on disc. Then the plates were incubated at 35-37°C for 12 to 18 hours.

RESULT

The results of the experiments carried out on the antimicrobial effect on the plant *Manilkara zapota* seeds extract with solvent methanol against clinical pathogens. Antibacterial activity of methanolic extracts of seeds *manikara zapota* at different concentrations (50,100,200 ppm disc) against different clinical pathogens with control was shown the following table.

Sr.no	Organism	Zone of inhibition (mm) Concentration in ppm		
		50	100	200
1.	<i>Escherichia coli</i>	15	16	17
2.	<i>Salmonella typhi</i>	13	16	18
3.	<i>Proteus mirabilis</i>	12	15	17

The seeds extract proved to be active against three different clinical pathogens strains such as *Escherichia coli*, *Salmonella typhi*, *Proteus mirabilis*. In this assay *Escherichia coli* was the most susceptible bacterium, on observation that may be attributed to the presence of Tannins alkaloids, inhibit the growth of microorganisms. The *Manilkara zapota* seeds extract, show highly active against the microorganisms. *E. coli* followed by *Salmonella typhi*, *Proteus mirabilis*, *Pseudomonas aeruginosa* in all the concentration.

CONCLUSION

Historically, medicinal plants have providing a source of inspiration for novel drug compounds, as plant derived medicines have made large gifts to human health and well being.

Seeds methanol extracts was found to be more effective against all microorganisms. The Aceton extracts was found to induce maximum inhibitory effect against all these microorganisms. In the seeds methanol extracts of *manilkara zapota* antibacterial activity was maximum in *Salmonella typhi* followed by others. In the flower methanol extract *manilkara zapota* antibacterial activity was maximum in *Escherichia coli* followed by others. This due to astringent antipyretic and also contain tannins. The results support earlier result as antibacterial activity of *Amry card power* (formulation) consists of *Manilkara zapota* against *E. coli*, *Escherichia coli*, *salmonella typhi*.

On the basis of the result obtained in this present study and conclude that the methanol extracts of *Manilkara*

zapota seeds had significant in vitro antimicrobial activity. and the most active extracts can be farther subjected to isolation and identify therapeutic antimicrobials and undergo further pharmacological evaluation.

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