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Evaluation and identification of phytochemical constituents and anti-microbial activity of *Manilkara zapota* leaves

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Abstract
Manilkara zapota leaves extracts were investigated for its bioactive compounds by screening phytochemically and quantitatively, along with characterization of the biocompounds using aqueous, petroleum ether, ethyl acetate, chloroform and ethanol extracts. Qualitative analysis revealed the presence of phenolic groups, flavonoids and triterpenoids in the ethanol and aqueous extract of the fruit. Total phenol content of the fruit in the aqueous and ethanol extract was 27.8 and 90.1 mg/100 gm, Flavonoid content was 39.3 and 106 mg/100 gm and Total triterpenoid was 49.9 and 61.9 mg/100 gm respectively. Antioxidant potential of *M. zapota* leaves extracts estimated through the DPPH radical scavenging activity and superoxide anion scavenging activity showed variation in the reduction potential. Antioxidant activity of the extracts estimated through the DPPH radical scavenging activity at different concentrations of 20, 40, 60, 80 and 100 µg /ml showed higher reduction ability at increasing concentration. The IC50 value through DPPH assay of the ethanol extract (86.2 µg /ml) followed by aqueous, ethyl acetate, chloroform and petroleum ether extract. Superoxide anion radical scavenging activity of *M. zapota* leaves extracts showed higher scavenging activity in methanolic extract. An IC50value of 74.62 µg /ml was shown by ethanolic fruit extract through Superoxide anion radical scavenging assay. The GC-MS analysis of methanolic leavesextract revealed the presence of 25 compounds which were eluted at various intervals of time. The compound 2-(Acetoxymethyl)-3-(Methoxycarbonyl) biphenylene (21st compound) showed the highest sharp peak 27.19 with the retention time (RT) of 16.516 minutes. Retention time (RT) of 8.526 minutes denoted the peak 0.22 which corresponded to the compound identified as Bicyclo (4.3.0) nonane, 3-methylene-.The work is discussed in detail.

Keywords: *Manilkara zapota*, phytochemical, antioxidant, DPPH

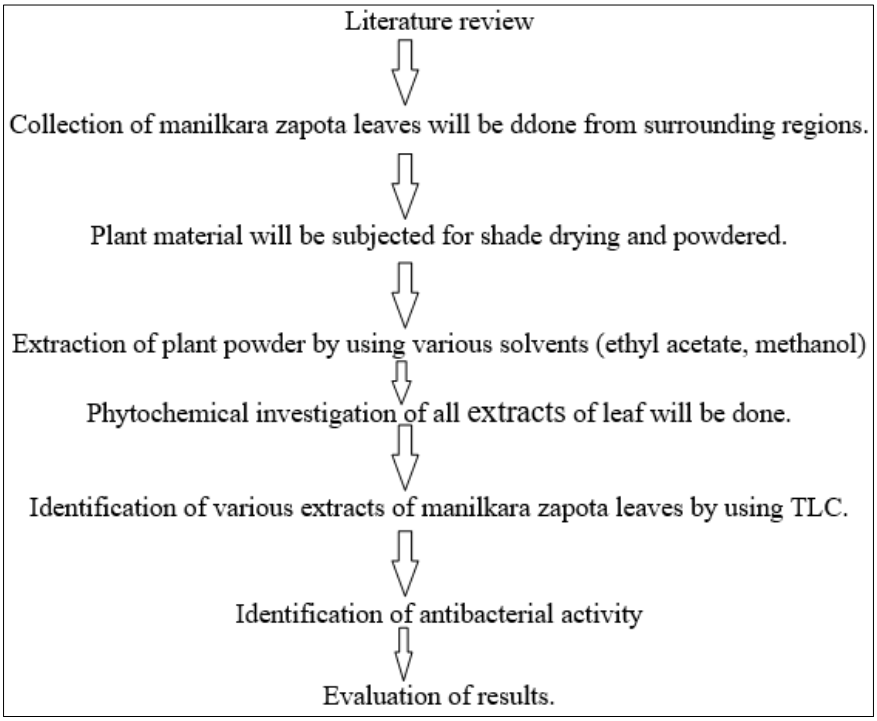
Introduction
Human companionship with the nature for food and shelter dates back to the origin of mankind. Healing power of the plants has been recognized by humans for thousands of years. Even today, more than 80% of the global population depends on traditional plant-based therapy for treating various diseases. Medicinal plants are defined as plants used for maintaining health and/or treating specific ailments. More than 9000 plants have been identified and recorded for their curative properties. There is a worldwide increase in the utilization of natural resources especially medicinal plants due to their perceived safety and efficacy, affordability and cultural acceptability. Medicinal effects of the plants are the result of the presence of different chemical compounds, also known as secondary metabolites, in the plant. Manilkara is a genus of trees in the family Sapotaceae consisting of ~79 species. *Manilkara zapota* (L.) P. Royen, commonly known as sapodilla, chiku and chicle, grows well in tropical conditions and is widely cultivated world over in tropical countries for various benefits like ediblefruits, timber, latex, etc. The fruit has an exceptionally sweet, malty flavour. Sapodilla fruit holds tremendous nutritional value as it is rich in sucrose and fructose. The fruit is consumed fresh or used to produce jams, compotes, and beverages. Traditionally *M. zapota* has been used for several medicinal purposes. All parts of the plants are ascribed to carry medicinal properties and are used for a range of disease including diarrhea, cold, fever and ulcers.

Scientific studies on *M. zapota* have revealed the presence of a wide range of bioactive constituents in this plant.

Aim

Screening and Characterization of Bioactive Compounds from *Manilkara zapota* leaves

Plan of work



Plant Profile

Current name: *Manilkara zapota*

Family: Sapotaceae

Common names

English: Chickle gum, Chicle tree, naseberry, sapodilla.

Manilkara zapota (Synonym: *Achras zapota* L.) is an evergreen canopy tree of medium size (15-30 meters in ht) native to Central America, which is currently cultivated throughout the tropics of the world (Castner *et al*., 1998) [13]. The Sapotaceae (Soapberry family) belongs to the Ebenales order along with the Ebenaceae, Styracaceae, Lissocarpacee, and Symplocaceae according to the Cronquist system of plant classification. Sapodilla, called *Manilkara zapota* originated in the central American rain forests but the crop has spread itself in India and in many other countries. The crop hasbecome the major commercial crop in India, Sri Lanka, Indonesia and Malaysia. Chewing gum has its origins in the economic botany of the Chicle tree (*M. zapota*). Throughout Mexico and Central America, the Sapotaceae plant family is recognized for its latex.

Fig 1: Leaves of *Manilkara zapota*

Materials and Methods

Collection of Material: Leaves of *Manilkara zapota* L collected from plants growing at sathupally surroundings. Telangana in India was used for this investigation. Fresh leaves were collected washed thoroughly and air dried in shade. After drying, the plant material was macerated using mixer grinder. Then the powder was stored in air tight containers and kept in refrigerator for future use.

Preparation of plant extracts

The dried leaves of *Manilkara zapota* were extracted with 10 grams of plant powder and 250ml of ethyl acetate, chloroform methanol and ethanol separated by using a soxhlet extractor for 8 hours and temperature not exceeding the boiling point of the solvent. The extracts were filtered using whatman (No 1) filter paper and then concentrates in vacuum at 40 degree Celsius using rotary evaporator. The residues obtained were stored in a freezer until further experiments.

Identification test: Thin layer chromatograph:

Procedure

1. With a pencil a thin mark is made at bottom of plate to apply the sample.
2. Then, sample solutions are applied on the spots marked on the line in equal distance.
3. The mobile phase is poured into the TLC chamber to a leveled few centri meters above the chamber bottom.
4. A moistened filter paper in mobile phase is placed on inner wall of the chamber to maintain equal humidity.
5. Sample line is facing the mobile phase, then the chamber is closed with a lid.
6. The plate is then immersed, such that the sample spots are well above the level of mobile phase for development.
7. Sufficient time is given for the development of spots.
8. The plates are then removed and allowed to dry.
9. The sample spots are then seen under uv light chamber.

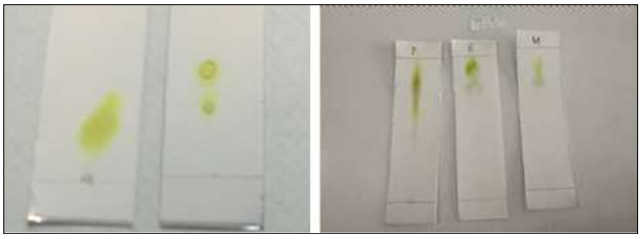


Fig 2: TLC plates of different mobile phases of Manilkara extract

The R_f value

The retention factor, or R_f, is defined as the distance traveled by the compound divided by the distance traveled by the solvent.

$$R_f = \frac{\text{distance traveled by the compound}}{\text{distance traveled by the solvent front}}$$

Travels 2.1 cm and the solvent front travels 2.8 cm, the R_f is 0.75:

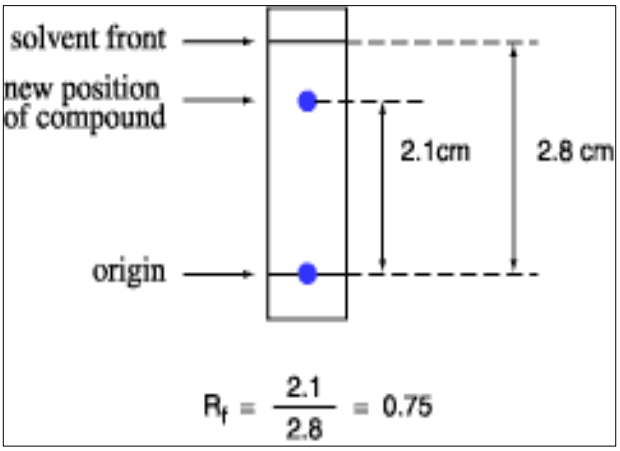


Table 1: Test for flavonoids

S. No	Test	Observation	Inference
01.	**General Test:** Alkaline reagent 2(or)3 drops of sodium hydroxide were added to2ml of extract then deep yellow colour was appeared but gradually becomes colourless by adding few drops of dil HCL.	Colourless	+
02.	**Shinoda's Test:** Alcoholic solution and mg metal (or) HCL were added to the extract then orange red (or) violet colour was produced.	Orange colour	+

Table 2: Test for Terpenoids

S. No	Test	Observation	Inference
01.	**Lupeol acetate test:** The dried crude extract (5mg) was dissolved in chloroform (2 ml) and acetic anhydride(1ml) and add conc.H2SO4 then reddish violet colour was appeared	Reddish colour	+
02.	**Salkowski Test:** The 5ml extract was mixed with chloroform (2 ml) and add conc.H_2SO_4 (3 ml) then reddish brown colour layer was appeared.	Brown colour	+

Antimicrobial Activity: Antimicrobial activity refers to the process of killing or inhibiting the disease causing microbes. Various antimicrobial agents are used for this purpose. Antimicrobial may be anti-bacterial, anti-fungal or antiviral. They all have different modes of action by which they act to suppress the infection.

Anti-bacterial identify by zone of inhibition

The Zone of inhibition is a circular area around the spot of the antibiotic in which the bacteria colonies do not grow. The zone of inhibition can be used to measure the susceptibility of the bacteria towards the antibiotics.

Preparation of agar media

Composition of nutrient agar:
It is solid medium and it is composition is
Agar-15 g
Peptone-5 g
Beef extract – 3 g
Nacl- 5 g
Distilled water -1000 ml

Preparation of nutrient media

1. Dissolve required quantity of chemical in 1000 ml distilled.
2. We put cotton plug in the mouth of conical flask and cover it with aluminum foil.
3. We sterilize the medium in autoclave for 15-20 minutes.

Serial dilution

1. We arrange the test tube and label it.
2. We add 1ml of zapota sample to the diluent.
3. We made further dilution.
4. Now, we perform plating from appropriately labelled petri plates after dilution.
5. 0.1µg sample to the petri plate.
6. Now, incubate all the plates at appropriate temperature and observe the colonies.

Result

We observe the inhibition of growth of bacteria at first, third, fourth, fifth.

S. No	Concentration	Zone of inhibition
01.	0.1 µg/ml	++
02.	0.2 µg/ml	_ _
03.	0.3 µg/ml	++
04.	0.4 µg/ml	_ _
05.	0.5 µg/ml	++
06.	0.6 µg/ml	++

Summary

Leaves of *Manilkara zapota* is commonly consumed, however in the present days this leaves is not given much importance. Hence it is the need of the hour to create awareness among the population about the medicinal property of the leaves of the plant. The plant with high bioactive potentials which can combat diseases, needs to be worked more. The active compounds present in the leaves extract were also characterized by GC-MS which has supported the medicinal property of the leaves. Moreover more bioactivity of these phytochemicals can be tapped further for drug designing for curing diseases of the present and future. There is also a worldwide increase in the use of herbal medicines as a complementary and alternative medicine to synthetic drugs once they are scientifically validated. *M. zapota* is a medicinal plant constituting versatile pharmacological profile and a wide range of compounds with diverse medicinal properties. This review, by providing a comprehensive understanding of pharmacological and chemical properties of *M. zapota*, put forth this plant as a candidate source with the potential to be explored for the discovery of new compounds with biological activities or as a validated complementary and alternative therapy through further laboratory and clinical investigations.

Conclusion

Our study provide additional data in which a total of 5 phyto-constituents have been identified in the *M. zapota* leaves aqueous extract that could be the contributors to their high antimicrobial contents and capacities. Therefore, our study suggests that *M. zapota* leaves is a good source naturally occurring antioxidants

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