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Screening And Characterization Of Bioactive Principles From Manilkara Zapota (L) P.Royen Fruits

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1. Abstract

Manilkara zapota (L) P. Royen fruit extracts were investigated for its bioactive compounds by screening phytochemically and quantitatively, along with characterization of the biocompounds usingaqueous, 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 27.85 and 90.1 mg/100gm, Flavonoid content was 39.3 and 106 mg/100gm and Total triterpenoid was 49.9 and 61.9 mg/100gm respectively. Antioxidant potential of M. zapota fruit extracts estimated through the DPPH radical scavenging activity and superoxide anion scavenging activity showed variation in their 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 IC_{50} 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 fruit extracts showed higher scavenging activity in ethanol extract. An IC_{50} value of 74.62 µg /ml was shown by ethanolic fruit extract through Superoxide anion radical scavenging assay. The GC-MS analysis of ethanolic fruit extract 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 peak27.19with the retention time (RT) of 16.516 minutes. Retention time (RT) of 8.526 minutes denoted the peak 0.22which 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, SARSA, GC-MS analysis

2. INTRODUCTION

Traditional medicine (also known as indigenous or folk medicine) comprises medical aspects of traditional knowledge that developed over generations within the folk beliefs of various societies before the era of modern medicine. The World Health Organization (WHO) defines traditional medicine as "the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness". From a long period of time medicinal plants or their secondary metabolites have been directly (or) indirectly playing an important role in the human society to combat diseases [1]. Secondary metabolites have no apparent function in a plants primary metabolism, but often have an ecological role as pollinator attractants represent chemical adaptations to environmental stresses of serve as chemical defense against microorganism insects and higher predated and even other plants (all chemicals). Secondary metabolites are frequently accumulated by plants in smaller quantities than the primary metabolites [2].

Pharmaceuticals used in modern medicine aspirn, atropine, ephedrine, digoxin, morphine, quinine, reserpine and tubocurarine serve as examples of drug discovered through observation of indigenous medical practices [3] Polyphenols are the most numerous and widely distributed class of phytochemicals many polyphenols, particulary the flavonoids, had been found to posses relativelypotent antioxidant, antiatheroscleortic, antitumour, antiinflammatory, antimutagenic and antiviral activities [4]. Phenolics are essential for growth and reproduction of plants and are produced as a response for defending against pathogens mainly during injury. It can enhance the body immune system to recognize and destroy cancer cells as well as inhibiting the development of new blood vessels that is necessary for tumour growth. Of the various classes of phytochemicals, interest has focused on the anti-inflammatory, and antioxidant properties of polyphenols found in various botanical agents [5].

3. Materials and Methods:

3.1.1 Collection of Plant Material:

Fruits of Manilkara zapota L collected from plants growing at Kudunkulam of Tirunelveli district of Tamil Nadu in India was used for this investigation. Fresh fruits 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.

3.1.2 Preparation of plant extracts

The dried fruits 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 [6].

3.1.4 Qualitative phytochemical test

The extracts of each solvent was used to analyze the presence of different phytochemical constituents. The method employed to analyze the phytochemical constituents were by following standard procedures.

3.1.5 Quantitative Analysis

Determination of flavonoids:

Total flavonoid content was determined by aluminium chloride methodusing catechin as a standard[7]. 1ml of test sample and 4 ml of water were added to a volumetric flask. After 5 min 0.3 ml of 5 % Sodium nitrite, 0.3 ml of 10% Aluminium chloride was added. After 6 min incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically.

3.1.5.2 Determination of Total phenols

The total phenolics content in different solvent extracts was determined with the Folin- Ciocalteu's reagent (FCR) using the procedure[7] of different concentrations of the 1 ml of the extract were mixed with 0.4 ml FCR . After 5 min 4 ml of 7% sodium carbonate solution was added. The final volume of the tubes were made upto 10 ml with distilled water and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer.

3.1.5.3 Determination of Terpenoids

Total terpenoid content was determined by the method of [8]. And the absorbance was read at 538 nm using UV/visible spectrophotometer.

3.2Determination of antioxidant activity of the extracts

3.2.1 Assay for Superoxide anion radical scavenging activity

The assay for superoxide anion radical scavenging activity was supported by riboflavin-light-NBT system [9].1 ml of extracts was taken at different concentrations (20, 40, 60, 80 and 100 µg/ml) and mixed with 0.1 ml of Riboflavin solution (20 µg), 0.2 ml of EDTA solution (12 mM), 0.2 ml of methanol and 0. 1 ml of Nitro-blue tetrazoliumn (0.5 mM) were mixed in test tube and reaction mixture was diluted up to 3 ml with phosphate buffer (50 mM). After 20 min of incubation at room temperature, the absorbance was measured at 560 nm. Ascorbic acid was used as standard. The scavenging ability of the plant extract was determined by the following equation:

Scavenging effect (%) = [(control OD − sample OD) / (control OD)] × 100

3.2.2 DPPH (2,2 Diphenyl-1-picryl-hydrazyl-hydrate scavenging assay)

DPPH assay was done following the procedure [9].An aliquot of 0.5 ml of sample solution in methanol was mixed with 2.5 ml of 0.5 mM methanolic solution of DPPH. The mixture was shaken vigorously and incubated for 30 minutes in the dark at room temperature. The absorbance was measured at 517 nm using UV spectrophotometer. Ascorbic acid was used as a positive control. DPPH free radical scavenging ability (%) was calculated by using the following formula.

$$\% \text{ of inhibition} = \frac{\text{OD of control} - \text{OD of sample}}{\text{OD of control}} \text{ X } 100$$

3.3 GC-MS Analysis[10]

GC-MS analysis of the ethanol extract of Manilkara zapotawas performed using a Perkin-Elmer GC Clarus 500 system comprising an AOC-201 auto sample and a gas chromatograph interfaced to a mass spectrometer (GC-MS) equipped with a Elite 5MS (5% biphenyl /95% dimethyl poly siloxance) fused a capillary column (30 X 0.25mm 1D X 0.25mm df). For GS-MS detection an electron ionization system was operated in electron impact mode with ionization energy of 70ev. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1ml/min, and an injection volume of 2ml was employed. The injector temperature was maintained at 200^0C, the ion source temperature was 200^0C, the oven temperature was programmed from 110^0 C, (isothermal for 2min), with an increase of 10^0C/min to 200^0C, then 5^0C/min to 280^0C, ending with a 9 min isothermal at 280^0C, mass spectra were taken at 70ev, a scan interval of 0.5S and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min, and the total GC-MS running time was 36min. the relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass detector used in this analysis was Turbo Mass Gold Perkin Elmer and the software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver-5.2.

4. RESULTS

4.1.1 Qualitative analysis of secondary metabolites from M. zapota fruit extracts

Manilkara zapota (sapotaceae), commonly known as sapodilla is one of the high-calorie fruits,100 g of the fruit provides 83 calories (almost same as that of calories in sweet potato, and banana). Additionally, it is an excellent source of dietary fibre (5.6 g/100g), which makes it a good bulk laxative. The fruits from M. zapota plants growing atKudunkulam in Tirunelveli district of Tamil Nadu, were subjected to phytochemical,quantitative, antioxidant, and GC-MS analysis.

The secondary metabolites of fruits of M. zapota plants growing at selected sites showed variation in their availability in the extracts analysed. Maximum phytochemicals were seen in the ethanolic extract of fruits which showed proteins, alkaloids, flavonoids, tannins, saponins, aminoacids and sugars. (Table 1)Qualitative analysis showed that phenol, flavonoid and triterpenoid was present in ethanol extract. Total phenol content of the fruit through aqueous and ethanol extract 27.85 and 90.1 mg/100gm. Flavonoid content displayed 39.3 and 106 mg/100gm. Total triterpenoid present in the sample exhibited 49.9 and 61.9 mg/100gm. Antioxidant potential of M. zapota extracts estimated through the DPPH radical scavenging activity and superoxide anion scavenging activity showed variation in their reduction potential. The ethanolic extract showed good antioxidant DPPH potential of 60.06 %, followed by chloroform and petroleum ether extract (44.8 and 42.4%). In the aqueous and ethyl acetate extract the antioxidant activity came to a value of 52.8 and 47.9% at 100 µg /ml of the sample when compared with standard ascorbic acid (Fig.4.1)**.** Superoxide anion radical scavenging activity of M. zapota fruit extract was ethanol ≥ aqueous ≥ petroleum ether ≥ ethyl acetate ≥ chloroform(Fig.4.2).

The lowest IC_{50} through DPPH assay was displayed minimal IC_{50} value of 86.2 µg /ml from ethanol extract followed by aqueous, ethyl acetate, chloroform and petroleum ether. While the lowest IC_{50} value through superoxide anion radical scavenging assay showed minimal concentration of 74.62 µg /ml in ethanol extract, followed by the other extracts analysed.

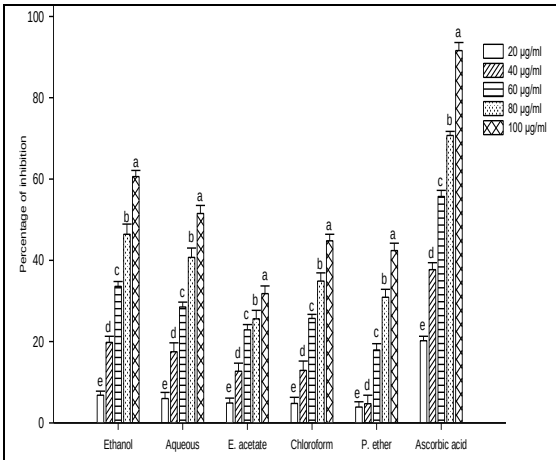

Fig 4.1. Antioxidant potential of M. zapota fruit by DPPH assay

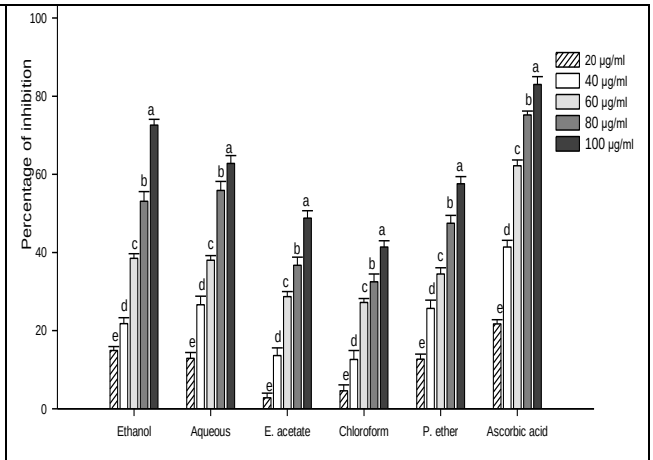

Figure 4. 2. Antioxidant potential of ethanolic extract of M. zapota fruit by superoxide anion radical scavenging assay

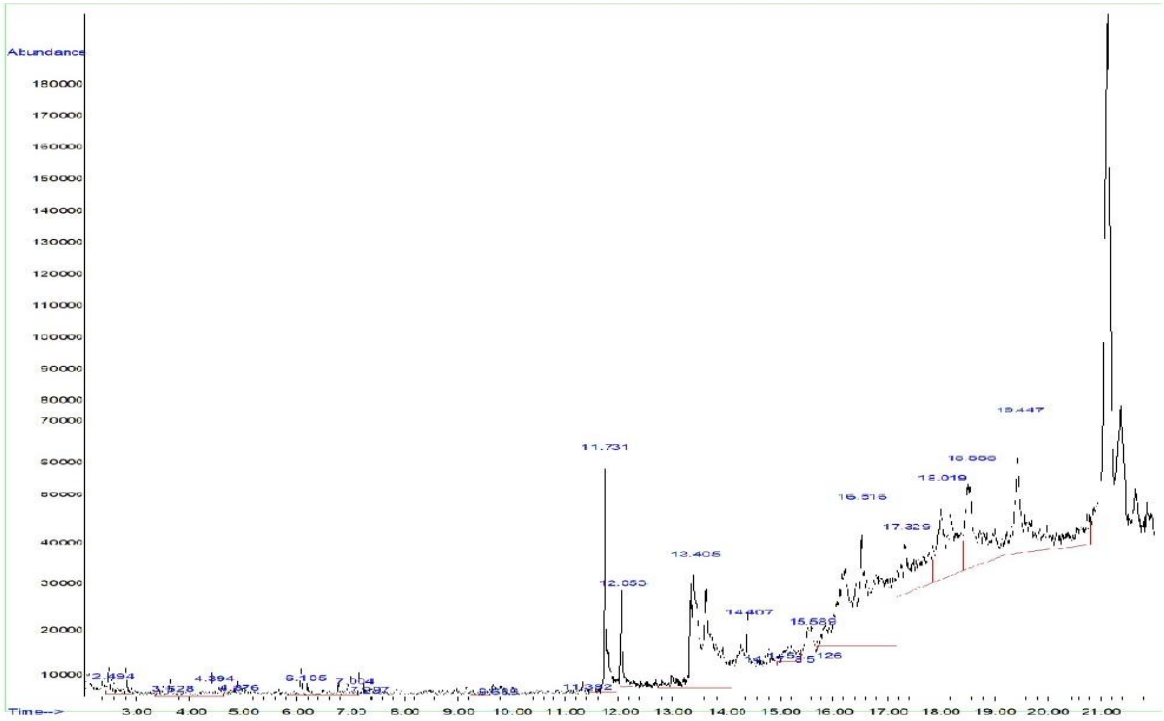

Fig. 4.3 GC-MS analysis of Manilkara zapota Fruits

4.3 GC-MS ANALYSIS

The GC-MS analysis of ethanolic fruit extract revealed the presence of 25 compounds distributed which were eluted and characterized. The compounds confirmed by NIST library were 1-Octanol, 2-butyl, Camphene, Tridecane, Hexadecane, 1,2-Pentadiene, (E,E)-7,11,15-Trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene, trans,cis-2,6-Nonadien-1-ol, 3-Ethyl-3-methylheptane, Bicyclo[4.3.0]nonane, 3-methylene, 2-Propyn-1-amine, N-methyl, 7-Oxabicyclo[4.1.0]heptane, 3-oxiranyl, 1,9-Nonanediol, dimethanesulfonate, Methyl 2,3-di-O-acetyl-4,6-di-O-methyl-.alpha.-D-mannopyranoside, n-Hexadecanoic acid, 2H-1-Benzopyran-2-one, 5,7-dimethoxy, 9,17-Octadecadienal,(Z), 7H-Furo[3,2-g][1]benzopyran-7-one, 4,9-dimethoxy-, [1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester, 1,2-Dihydroanthra[1,2-d]thiazole-2,6,11-trione, Silicic acid, diethyl bis (trimethylsilyl) ester, 2-(Acetoxymethyl)-3-(methoxycarbonyl) biphenylene, 4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene) tyramine, 1,2-Bis(trimethylsilyl)benzene, Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl and 2-Ethylacridine. The result revealed the presence of the compound 2-(Acetoxymethyl)-3-(methoxycarbonyl) biphenylene (21st compound) had the highest sharp peak 27.19 with the retention time (Rt) of 16.516 minutes. Retention time (Rt) of 8.526 minutes denoted with the peak 0.22 which had a 9 th compound identified as Bicyclo (4.3.0) nonane, 3-methylene- (Fig.4.3, Table2).

5. DISCUSSION

Plants are endowed with phytochemicals, which are synthesized, stored and available in various parts such as leaves, stem, bark, roots etc. Thus plant based extracts exhibit more medicinal property in its natural environment.Phytoconstituents extracted from the spices, herbs, plants are reported to have anti-inflammatory, antidiarrheal, antimicrobial, antioxidant and insecticidal activities [11]. More of the presentday research is engaged in finding novel compound and their biological properties, pharmacological actions etc. The availability of secondary metabolites in plants are responsible for various biological activity and their nutritional value. The qualitative analysis of M. zapota fruits extracted through petroleum ether, chloroform and ethanol showed presence of many metabolites. The ethanolic extracts of the fruits showed presence of steroids, triterpenoids, reducing sugars, phenolic groups, proteins, alkaloids, flavonoids, tannins, saponins, aminoacids and sugars. The earlier reports showed glycosides, phenolic compounds and terpenoid compounds in M. zapota fruit extract [12]. The presence of active phytochemical constituents such as alkaloids, flavonoids, phenol, tannins, saponins, terpenoids and others are the basic reasons for a plant to exhibit medicinal activity [13].The presence of flavonoids in ethanolic extract could led to contributing to the better antioxidant property shown by plants[14]. The analysis of total phenols, flavonoids and triterpenoids in the fruit extracts of ethanol showed that the availability of these phytoconstituents was higher in fruits. The flavonoid content was higher than phenols and

triterpenoids in this work. Research report from earlier researchers on M. zapota fruit peel extracts revealed quantitative estimation of phenolic compounds, flavanoids, carotenoids [15]. The qualitative analysis of M. zapota fruit ethanol extracts revealed more availability of phytoconstituents which implies with its antioxidant property. This can be corroborated with the fact that alkaloids, flavanoids, terpenoids and steroids compounds are also well known to have potential antioxidant activity[17,18], The flavonoids and phenols are known to have antiradical and antioxidant activity [16]. Earlier report reveals M. zapota fruit peel has antioxidant containing many important bioactive phenolic compounds which has promising health benefits [19].

The DPPH and superoxide assay of ethanolic fruit extract of M. zapota revealed good scavenging property showing low IC_{50} values in the tested concentrations, which can be corroborated with the phytochemical and quantitative content of biomolecules done in this investigation. The present work also agrees with, plant polyphenols act as reducing agents and antioxidants by the hydrogen-donating property of their hydroxyl groups [20]. Hence it can beconclude that these polyphenols are responsible for the observed antioxidant activity in this study.The antioxidant activity of phytochemicals are predominantly determined by their electron delocalization over an aromatic nucleus, in those based on a phenolic structure [21]. The polyphenols which includes the different flavonoids like flavonols, flavones, isoflavones, and anthocyanidins), stilbenes, lignans, and phenolic acids [22], have the ability to react with a free radical and the scavenging action results in the delocalization of the gained electron over the phenolic antioxidant and the stabilization by the resonance effect of the aromatic nucleus, which averts the continuation of the free radical chain reaction [21]. Earlier report revealed the acetone extract of M. zapota leavesexhibited DPPH and superoxide and hydroxyl radical scavenging activity showed good antioxidant capacity, this implies the intake of food in our daily supplements foods conferring protection against oxidative damage [23].

The gas chromatography mass spectrum revealed25 compounds were identified in the ethanol fruit extract of M. zapota. 1-Octanol, 2-butyl eluted at Rt 2.49, the compound which was studied by earlier researcher revealed it has good antimicrobial activity, the same compound was also identified in ethanolic leaf extract of Feronia elephantum[24].

The compound camphene recognized from M. zapotafruit extract exhibit antifungal, antidyslipidemic and anti-inflammatory activity[25].Tridecane identified in fruit extract has more medicinal values like good antimicrobial activity against antibacterial activity against Acetobacter calcoacetica, Bacillus subtillis, Clostridium sporogenes, Clostridium perfringens, Escherichia coli, Salmonella typhii, Staphylococcus aureus and Yersinia enterocolitica strains [26].

Hexadecane- which helps to accelerate biological process [27]. suggests that hexadecane helps to stimulate lipase production needed for the process. Bicyclo [4.3.0]nonane, 3-methylene identified from M. zapota fruits was also identified from methanolic extracted Callosobruchus maculutus, which showed good antifungal activity against most strains such as Aspergillus niger, Aspergillus terreus, Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Saccharomyces cerevisiae, Penicillium expansum and Trichoderma viride [28]. Methyl 2,3-di-O-acetyl-4,6-di-O-methyl-.α.-D-mannopyranoside a monosaccharide derivative which was identified in this workis known to have antibacterial good activity [29].The compound n-Hexadecanoic acid, from M. zapota has more biological properties they are good for anti-Inflammation [30], treatment of rheumatic symptoms [31] hypocholesterolemic, nematicide, anti-androgenic, hemolytic, pesticide, lubricant, 5-alpha reductase inhibitor, antipsychotic.

Identified compound 9,17-Octadecadienal, (Z) from M. zapotaand earlier researchers reveals that the compound exhibit antimicrobial property [32], antiflammatory [33]. Compound 7H-Furo [3,2-g][1]benzopyran-7-one, 4,9-dimethoxy present in M. zapota was also detected from leaves of Chloroxylon swietenia,is used to produce eco-friendly gold nanoparticles synthesized showing larvicidal activity against malarial vector, Anopheles stephensi and dengue vector, Aedes aegypti [34].

Silicic acid, diethyl bis (trimethylsilyl) ester identified has some biological properties antimicrobial activity, the same compound was also identified from Haliclona sp [35], which was extracted through diethyl ether subjected to GC-MS revealed presence [36]. 2-(Acetoxymethyl)-3-(methoxycarbonyl) biphenylene- compound present in M. zapota can also be produced by Staphylococcus vitulinus which was identified through GC-MS [37].2-Ethylacridine compound present in ethanol extract of M. zapota also found in acetone and chloroform extract of Dicranopteris linearis[38]. Inner bark of Berberis vulgaris stem showed its presence, and the extract showed good antioxidant potential through DPPH assay [39].

6. CONCLUSION

Fruits of Manilkara zapotais commonly consumed, however in the present days this fruit 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 fruit of the plant. The plant with high bioactive potentials which can combat diseases, needs to be worked more. The active compounds present in the fruit extract were also characterized by GC-MS which has supported the medicinal property of the fruit. Moreover more bioactivity of these phytochemicals can be tapped further for drug designing for curing diseases of the present and future.

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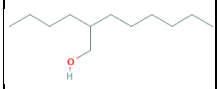
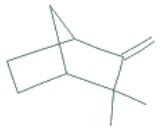
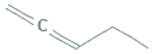
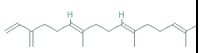
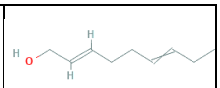
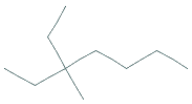
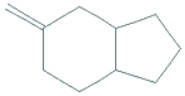
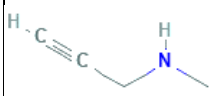
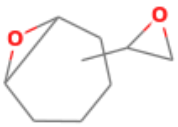
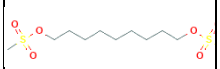
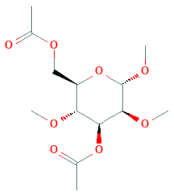
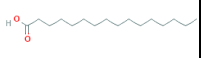
Table 1 Qualitative phytochemical analysis of M. zapota fruitextract from Tirunelveli

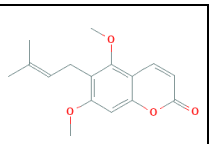
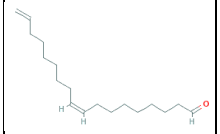
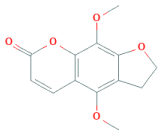
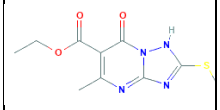
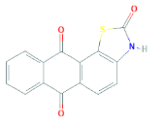
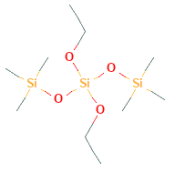
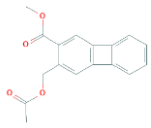
Sl No	Test	Aqueous	Petroleum Ether	Ethyl Acetate	Chloroform Extract	Ethanol Extract
1	Steroids	-	-	+	+	+
2	Triterpenoids	+	+	+	-	+
3	Reducing Sugar	+	+	+	+	+
4	Phenolic Groups	+	-	-	-	+
5	Proteins	-	-	-	-	+
6	Alkaloids	-	-	-	-	+
7	Flavonoids	+	-	-	-	+
8	Catechin	-	-	-	-	-
9	Tannins	+	-	-	-	+
10	Anthroquinones	-	-	-	-	-
11	Saponins	-	-	-	-	+

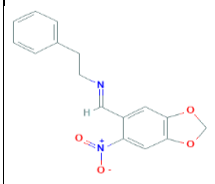
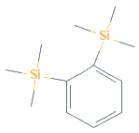
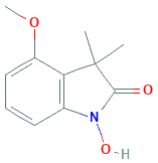
12	Aminoacids	-	-	-	-	+
13	Sugars	+	-	+	+	+

Table 2. Compounds identified from ethanolic fruit extract of M. zapota using Gas chromatography mass spectrum.

Sl. No	Compound Name	Retention time	Area %	Molecular weight(g/ mol)	Molecular formula	Structure
1	1-Octanol, 2-butyl-	2.49	1	186.33	$C_{12}H_{26}O$	
2	Camphene	3.62	0.46	136.23	$C_{10}H_{16}$	
3	Tridecane	4.39	0.99	184.36	$C_{13}H_{28}$	
4	Hexadecane	4.87	0.66	226.44	$C_{16}H_{34}$	
5	1,2-Pentadiene	5.68	0.29	68.12	C_5H_8	
6	(E,E)-7,11,15-Trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene	6.10	0.55	272.5	$C_{20}H_{32}$	

7	trans,cis-2,6-Nonadien-1-ol	7.00	1.12	140.22	$C_9H_{16}O$	
8	3-Ethyl-3-methylheptane	7.29	0.3	142.28	$C_{10}H_{22}$	
9	Bicyclo[4.3.0]nonane, 3-methylene-	8.52	0.22	136.23	$C_{10}H_{16}$	
10	2-Propyn-1-amine, N-methyl-	9.53	0.27	69.11	C_4H_7N	
11	7-Oxabicyclo[4.1.0]heptane, 3-oxiranyl-	9.68	0.91	140.180	$C_8H_{12}O$	
12	1,9-Nonanediol, dimethanesulfonate	10.5	0.31	316.4	$C_{11}H_{24}O_6S_2$	
13	Methyl 2,3-di-O-acetyl-4,6-di-O-methyl-.alpha.-D-mannopyranoside	11.38	0.64	306.31	$C_{13}H_{22}O_8$	
14	n-Hexadecanoic acid	11.73	4.29	256.42	$C_{16}H_{32}O_2$	

15	2H-1-Benzopyran-2-one, 5,7-dimethoxy-	12.05	1.95	274.31	$C_{16}H_{18}O_4$	
16	9,17-Octadecadienal, (Z)-	13.40	15.09	264.4	$C_{18}H_{32}O$	
17	7H-Furo[3,2-g][1]benzopyran-7-one, 4,9-dimethoxy-	14.40	2.17	246.21	$C_{13}H_{10}O_5$	
18	[1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester	14.78	0.54	268.29	$C_{10}H_{12}N_4O_3S$	
19	1,2-Dihydroanthra[1,2-d]thiazole-2,6,11-trione	15.12	1.21	281.29	$C_{15}H_7NO_3S$	
20	Silicic acid, diethyl bis(trimethylsilyl) ester	15.58	1.19	296.585	$C_{10}H_{28}O_4Si_3$	
21	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	16.51	27.19	282.29	$C_{17}H_{14}O_4$	

22	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine	17.32	6.72	298.29	$C_{16}H_{14}N_2O_4$	
23	1,2-Bis(trimethylsilyl)benzene	18.01	9.07	222.47	$C_{12}H_{22}Si_2$	
24	Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-	18.55	9.35	207.23	$C_{11}H_{13}NO_3$	
25	2-Ethylacridine	19.44	13.5	207.27	$C_{15}H_{13}N$