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Phytoconstituents from the leaves and seeds of *Manilkara zapota* Linn

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ABSTRACT

*Bioassay guided isolation of crude methanolic extracts of the leaves and seeds of M. zapota yielded a total of three known compounds, myricetin-3-O-α-L-rhamnoside (**1**), D-quercitol (**2**) and saccharose (**3**). The structure of the compounds were established based on their physical and chemical data (^{1}H, ^{13}C NMR, 2D NMR and mass). The compound (**1**) showed good antioxidant activity, moderate elastase and tyrosinase inhibition activities.*

Key words: *Manilkara zapota*, chemical constituents, biological studies**.**

INTRODUCTION

Recently the secondary metabolites isolated from the medicinal plants have been receiving good attention for several cosmetic applications, Ex. Skin lightening through mushroom tyrosinase inhibition,[1] melanin synthesis inhibition[2] and dendrite elongation inhibition,[3] anti-aging by matrix metalloproteinase enzyme inhibition,[4] skin rejuvenation by anti-oxidant mechanism,[2] hair growth by 5α-reductase mechanism, [5] and antidandruff by fungal growth inhibition[6] etc. There exists several reports on the ability of plant extracts either singly or in combinations serving as cosmetic applications such as *Ramulus mori*, [7] *Glycyrrhiza glabra*,[8] *Artocarpus heterophyllus*, *Piper longum* etc for skin lightening, *Mangifera indica and Psidum guajava* for anti-aging,[9] green tea extract for protecting against free radical damage, *N. jatamansi* extract for hair growth,[11] and *Aerva sanguinolenta* extract for antimicrobial activity[12] etc. Scientists are showing more interest on natural extracts which can help and protect from various disease. Few potent active compounds were isolated and characterized for various cosmetic applications. These are mulberrin [7] and isoliquiritigenin [8] for skin lightening, triterpenes for elastase inhibition activity, [9] lupeol for melanin promotion activity,[10] nardin for hair growth activity,[11] bakuchiol for antibacterial activity.[12]

Based on our continuous interest on the isolation and characterization of bioactive secondary metabolites from Indian medicinal plants for personal care applications,[13] we have undertaken the leaf and seeds of *Manilkara zapota* Linn., for chemical and biological studies. *Manilkara zapota* commonly known as the Sapodilla, is a long-lived, evergreen tree native to southern Mexico, Central America and the Caribbean.[14] It was introduced to the Philippines during Spanish colonization. It is grown in huge quantities in India, Thailand, Malaysia, Cambodia, Indonesia, Bangladesh and Mexico.The riped fruit has an exceptionally sweet, malty flavor. The unripe fruit is hard to the touch and contains high amounts of saponin, which has astringent properties similar to tannin, drying out the mouth. Acetone extracts of the seeds exhibited considerable antibacterial effects against strains of *Pseudomonas oleovorans* and *Vibrio cholerae*.[15]

Compounds extracted from the leaves showed anti-diabetic, antioxidant and hypocholestero-lemic (cholesterol-lowering) effects in rats.[16] Two new antioxidant active compounds, methyl 4-O-galloylchlorogenate and 4-O-galloylchlorogenic acid along with eight known compounds were reported from the methanolic extract of fruit of *M. zapota*.[17] The plant grown in Egypt showed different constituents, unsaturated fatty acids, oleic acid, linolenic acid and linoleic acid, lupeol acetate, oleanolic acid, apigenin-7-O-a-L-rhamonoside, myricetin-3-O-α-L-rhamonoside and caffic acid. The extract from the leaves exhibited anti-hyperglycemic, hypochlolesterolemic and antioxidant activities.[16]

In this paper we wish to report the isolation and identification of three known compounds from the methanolic extracts of leaves and seeds of *Manilkara zapota* and its biological activities: DPPH activity, Tyrosinase inhibition activity and elastage inhibition activity. These structures were identified based on physical and chemical data and comparison with literature data.

MATERIALS AND METHODS

General procedures

Melting points were reported uncorrected. IR spectra were recorded on a Shimadzu Prestige 21 FT IR. UV spectra were recorded on Shimadzu UV spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker AMX 400 with TMS as an internal standard. Mass spectra were recorded on Jeol SX 102/DA 600 mass spectrometer. Column chromatography (CC) was carried on a silica gel column (100-200 mesh). Purity of the samples was checked by TLC on pre-coated aluminum sheets, silica gel 60 F_{254} (20 X 20 cm, 0.2 mm thickness, Merck) and compounds were detected under UV light (254 & 366 nm) and spraying with 5% sulphuric acid in methanol followed by heating the plates at 110^{o}C for 5 min. The chemical shift values are reported in ppm (δ) units and the coupling constants (*J*) are in Hz. The standard compounds were obtained from M/s. Sigma Aldrich, USA.

Plant material

The leaves and seeds of *M. zapota* Linn., were obtained from local market in Tamil Nadu in January 2011. The taxonomical identification was done by Dr. P. Santhan, taxonomist, M/s. Durva Herbal Centre, Chennai, Tamil Nadu, India. All voucher specimen were deposited in M/s. CavinKare Research Centre, Chennai, India.

Extraction and Isolation of compounds

The dried leaves of *M. zapota* Linn., (700 g) were coarsely powdered, extracted with methanol (3.0 L) by using soxhlet apparatus. The dilute extract was concentrated by rotary evaporator under reduced pressure at ~40^{o}C to get 210 g crude methanolic extract. The methanolic extract was showed mushroom tyrosinase inhibition activity (39% at 100 µg/ml) and antioxidant activity (88%at 40µg/ml). Part of the extract (200 g) was suspended in water: methanol (9:1) and fractionated with hexane, chloroform, ethyl acetate and aq. residue to get corresponding fractions 30.7g, 4.5g, 26.8, and 120g respectively. The ethyl acetate fraction showed more potent activity than other fractions. The ethyl acetate fraction was further purified by small silica gel followed by sephadex LH-20 column and finally crystallization with ethyl acetate:acetone (8:2), obtained as pale yellow colored solid and was identified as myricetin-3-O-α-L-rhamnoside (**1**, 120 mg).[18]

The seeds of *M. zapota* Linn., (500 g) were coarsely powdered, extracted with methanol (2.0 L) by using soxhlet apparatus for about 8 hrs. The dilute extract was concentrated under reduced pressure by using rotary evaporator at ~ 40^{o}C to get 60 g crude methanolic extract. Part of the extract (20 g) was adsorbed on silica gel (60-120 mesh) and chromatographed over silica gel using chloroform: methanol (8:2, 6:4, 4:6) to get a total of 10 fractions. Each fraction volume is 250 ml. Combined homogeneous fractions based on TLC and made into three major fractions, Fr.1 (6.5g), Fr. 2 (0.35 g) and Fr. 2 (0.95g). Fraction 2 was found to be solid nature and showed single spot on TLC, which was crystallized with methanol to get colorless solid and identified as D-quercitol (**2**, 120mg).[19] Fraction 3 was also showed solid nature on concentration, which was crystallized with water: methanol to get colorless solid and identified as saccharose (**3**, 882 mg).[13] All three compounds were submitted for tyrosinase inhibition, elastase inhibition and antioxidant property. Compound **1** was showed potent antioxidant property than other two compounds. The same compound showed moderate elastase and tyrosinase inhibition activities where as other two compounds showed no activity.

Compound 1: Pale yellow color solid; mp:185^{o}C**;** UV (MeOH) nm : 254, 273, 303, 360 nm; ^{I}H NMR (400MHz, DMSO): δ 0.83 (3H, d, J=6.1Hz, H-6"), 3.14 (1H, t, J=9.4Hz), 3.42 (1H, m), 3.52 (1H, dd, J=3.0, 9.3Hz), 3.97 (1H, 1H, s), 5.18 (1H, s, H-1"), 6.19 (1H, d, J-1.9Hz, H-6), 6.35 (1H, d, J=1.9Hz, H-8), 6.88 (2H, s, H-2',6'), 9.0-9.5 (2H, br, OH), 12.68 (1H, br, OH-5); ^{13}C NMR (100MHz, DMSO): δ 17.9, 70.4, 70.7, 70.9, 71.6, 93.9, 99.0, 102.3, 104.4, 108.2, 119.9, 134.6, 136.8, 146.1, 146.1, 156.8, 157.8, 161.6, 164.6, 178.1. EIMS m/z: 464.

Compound 2 (Fig 1): Colorless solid; mp: 222^{o}C**;** ^{I}H NMR (400MHz, DMSO): δ1.60 (2H, m), 3.27 (1H, t, J=8.9Hz), 3.38 (1H, dd, J=3.0, 9.2 Hz), 3.45 (1H, m), 3.58 (1H, t, J=3.1Hz), 3.67 (1H, d, J=3.3Hz); ^{13}C NMR (100MHz, DMSO): δ 34.5, 68.4, 69.0, 71.3, 72.7, 74.9, EIMS m/z: 148.

Compound 3 (**Fig 1**): Colorless crystals, mp: 271-272^{o}C; UV (MeOH) nm: end absorption; IR (KBr) cm^{-1}: 3322 (br), 1066 and 835cm^{-1}; ^{1}H NMR (400 MHz, D_2O): δ5.17 (1H, d, J=3.8 Hz) 3.98 (1H, d, J=8.8 Hz), 3.81 (1H, t, J= 8.6Hz), 3.70-3.60 (2H, m), 3.60-3.55 (4H, m), 3.51 (1H, t, J=11.4Hz), 3.43 (2H, s), 3.30 (1H, dd, J=3.8, 13.8 Hz), 3.25 (1H, t, J=18.4 Hz); ^{13}C NMR (100 MHz, D_2O) : δ 59.9, 61.1, 62.2, 69.0, 70.6, 72.2, 72.3, 73.8, 76.2, 81.2, 92.0, 103.5; EIMS *m/z* : 342.

RESULTS AND DISCUSSION

The compound **1** obtained as pale yellow colored solid, its UV spectrum showed 254, 273, 303, 360 nm indicating that the compound may be having flavanone basic skeleton. The IR spectrum showed peaks at 3350cm^{-1} (br, hydroxyl), 1705cm^{-1} (C=O), 1690 and 1620 cm^{-1} (aromatic). The ^{1}H NMR spectrum showed the presence of two meta coupled aromatic protons at δ 6.19 (1H, d, J=1.9Hz, H-6), 6.35 (1H, d, J=1.9Hz, H-8), two aromatic protons at δ 6.88 (2H, s, H-2',6'), three phenolic hydroxyl peaks at δ 9.0-9.5 (2H, br, OH), 12.68 (1H, br, OH-5). Additionally, the spectrum showed the presence of methyl at δ0.83 as doublet, five hydroxyl bearing protons at δ 3.14 (1H, t, J=9.4Hz), 3.42 (H, m), 3.52 (1H, dd, J=3.0, 9.3Hz), 3.97 (1H, 1H, s) and an anomeric proton appeared at δ5.18 as singlet indicating that compound is having rhamnoside group attached to the flavonone. The carbon spectrum clearly showed the presence of 21 carbons signals, of which six sugar carbons (δ 17.9, 70.4, 70.7, 70.9, 71.6, 102.2) and remaining 15 belongs to flavones moiety. By revealing the literature, the spectral data of the compound **1** is exactly matching with those of previously reported values of myricetin-3-O-α-L-rhamonoside or myricitrin.[16]

The compound **2** came as colorless solid from methanol:chloroform. It was readily recognized as simple hydroxyl compound based on its NMR spectral data. Its molecular formula has been fixed $C_6H_{12}O_4$ based on its mass spectrum. Its UV spectrum showed end absorption. The IR spectrum showed the peak at 3200-3400cm^{-1} indicating for hydroxyl group. The proton NMR spectrum clearly showed the presence of five oxygenated protons at δ 3.67 (1H, d, J=3.3Hz), 3.58 (1H, t, J=3.1Hz), 3.45 (1H, m), 3.38 (1H, dd, J=3.0, 9.2 Hz), 3.27 (1H, t, J=8.9Hz) and one methylene group at δ 1.60 (m). The carbon spectrum clearly showed a total of six carbon peaks. Of which, five oxygenated carbons at δ 74.9, 72.7, 71.3, 69.0, 68.5 and one methylene carbon at δ 34.5. The mass spectrum showed the molecular ion at 164. Based on the above data, a literature search revealed that the physical and spectral data of the compound **2** agreed perfectly with D-quercitol and also direct comparison with an authentic sample reported by our laboratory.[19]

Tyrosinase inhibition: Tyrosinase inhibition activity was determined by dopachrome method using L-tyrosinase as substrate. Fresh solutions of L-tyrosine (3 mM), buffer (pH 6.8, 50 mM) and a stock solution of 500units/mL of mushroom tyrosinase were prepared. The reaction mixture constituents 235 µL of L-tyrosine, 365 µL of buffer and 90 µL of enzyme and 10 µL of inhibitor. The assay mixture was incubated at 37^{o}C for 30 min. The dopachorme was measured spectrophotometrically at 475nm and % of inhibition was calculated. The results of the crude methanolic extract, standard compound, kojic acid and compound **1** were given in the table 1.

Elastase inhibition: The elastase inhibition activity of crude extract, different fractions and compounds along with ursolic acid were studied on cell free system. The assay method is most reliable method and reported in the literature.[20] Fresh solution of 300 µl (0.6 mg) of succinyl-L-alanyl-L-alanyl-L-alanyl-p-nitroanilide (enzyme substrate), 1200 µl of buffer and varying amounts of the elastase inhibitor under testing are incubated at 37^{o}C for 20 minutes. The hydrolysis is measured by the spectrophotometric measurement of the release of p-nitroaniline at a

wavelength of 410 nm. The crude methanolic extract, fractions and isolated compound were tested and results were mentioned in the table 1.

Antioxidant activity (DPPH method): The antioxidant activity of crude extract and the isolated compound along with ascorbic acid were studied on cell free system. The assay method has been reported in the literature by many scientists. The crude methanolic extract, fractions and isolated compound were tested and results were mentioned in the table 1.

Table 1: Comparative evaluation of crude extract, fraction and isolated compound

Sl.No	Parameter	Tyrosinase inhibition(µg/ml)	Elastase inhibition(µg/ml)	Anti-oxidant activity (µg/ml)
1	Crude methanolic extract of Leaf	39% at 40	72% at 40	79% at 40
2	Ethyl acetate fr from leaf extract	58% at 40	60%at 40	88% at 40
3	Compound **1**	30% at 100	34% at 40	94% at 40
4	Kojic acid	IC_{50}= 1.73	-	-
5	Ursolic acid	-	IC_{50}= 13.1	-
6	Ascorbic acid	-	-	IC_{50}= 2.4

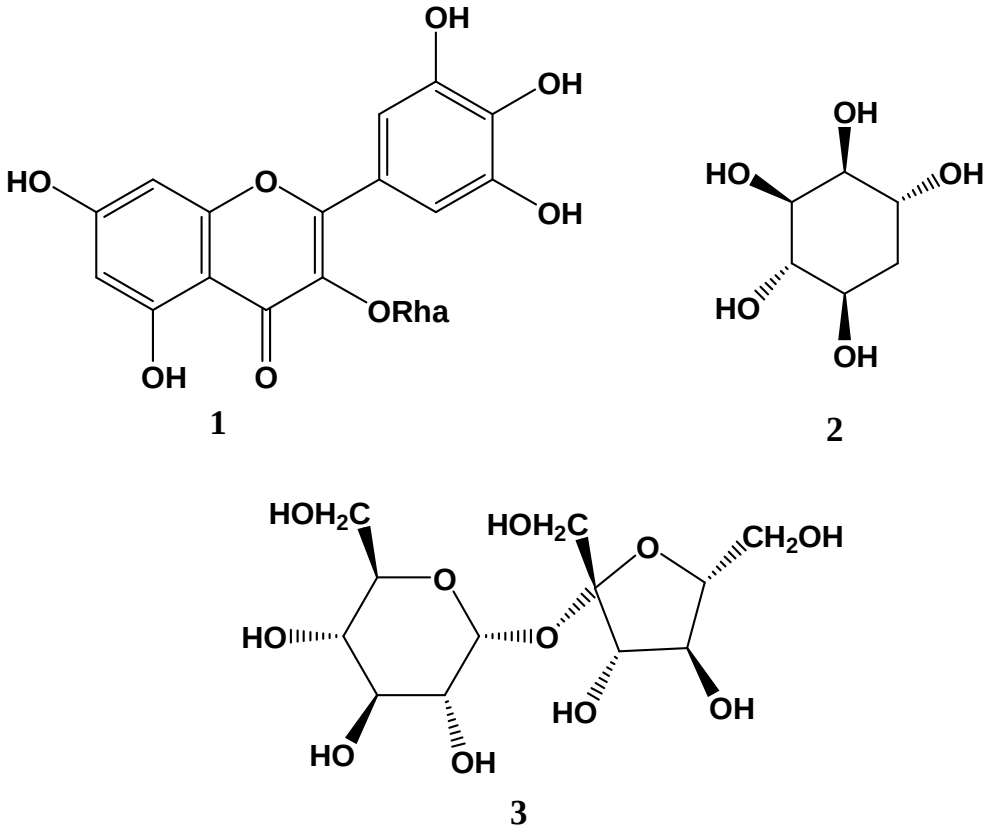

Figure: Compounds from *Manilkara zapota* Linn.

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