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Secondary metabolites from the flowers of *Mimusops elengi* Linn.

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ABSTRACT

*The air dried flowers of folklore medicinal plant, Mimusops elengi Linn., yielded four known compounds: Oleanolic acid (**1**), 4-hydroxybenzaldehyde (**2**), stigmasterol-3-O-β-D-glucopyranoside (**3**) and D-quercitol (**4**). Structure of the compounds were elucidated on the basis of spectroscopic analysis (IR, ^{1}H & ^{13}C NMR and Mass) and Co-TLC comparison with an authentic samples and literature data. One of the compound was reported for the first time from this plant. The methanolic extract and isolated compounds were examined for in-vitro elastase inhibition and also melanin promotion activities.*

Keywords: *Mimusops elengi*, chemical constituents, biological studies.

INTRODUCTION

Mimusops elengi Linn., is an ever green tree found in the Deccan Peninsula and Andaman Islands and cultivated in gardens for ornamental purpose. The bark is acrid and sweet. It is being used as anthelmintic, astringent, stomachic, cures biliousness and diseases of the gum and teeth.[1] The flowers are sweet, acrid and are used as expectorant, cures diliousness, liver complaints, dieases of nose, headache, tooth problems and smoke is good for control asthma.[1] The leaves are known for analgesic and antipyretic.[2] In Thailand, an infusion of the flowers is used as a cosmetic after bath. The ripe fruit is edible and some times used for making preserves and pickles. A snuff made from dried flowers induces copious defluxion from the nose and relieves headache and pain.[3] Various parts of the plant, *M. elengi* showed different kinds of biological and pharmacological activities, like antibatcterial, anti-fungal, anti-carcinogenic, free radical scavenging, anti-hyperglycemic, anti-viral anti-neoplastic, anti-noceptive, diuretic and gastroprotective effects.[1] Previous reports on this plant occurring in different regions yielded variety of compounds such as steroids,[4] terpenoids and their glycosides,[5] flavonoids,[6] sugars,[6] lipids, saponins and alkaloids.[3] The seed compound, mimusopic acid exhibited anti-HIV reverse transcriptase activity.[7] Two α-glucosidase inhibitors, 3β-hydroxy-12-ursene-28-oic acid and 3β-(4-hydroxycinnamoyl)-12-ursen-28-oic acid were reported from the methanolic extracts of *M. elengi*.[8]

In continuation of our interest on the isolation of biologically active molecules from medicinal plants for personal care applications,[9-16] we have undertaken the dried flowers of *M. elengi.* In this paper, we report the isolation and structure elucidation of four known secondary metabolites, **1-4** (Figure 1). Structure of the compounds were identified by NMR spectral data and compared with literature data and co-comparison with an authentic compounds..

MATERIALS AND METHODS

General Procedures:
IR: Prestige 21 FT IR (Shimadzu); UV: Shimadzu UV spectrophotometer; NMR: ^{1}H and ^{13}C NMR (Bruker AMX 400); Mass spectrum: 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, 0.2mm 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.

Plant material
The flowers of *Mimusops elengi* Linn., (430 kg) were obtained from Bazar in December 2007 and identified by Dr. P. Santhan, Toxanamist, Durva Herbal Centre, Chennai, Tamil Nadu, India. A voucher specimen of the species was deposited in M/s. CavinKare Research Centre, Chennai, India.

Extraction and Isolation:
The flowers of *M. elengi* Linn., (430 g) were exhaustively extracted with methanol (3.0 L) by using soxhlet apparatus. The solvent was removed by rotary evaporator under reduced pressure at ~ 40^{o}C to get 63.9 g crude methanolic extract. The methanolic extract showed melanin promotion activity in cell lines. The crude methanolic extract (37 g) was suspended in methanol: water (1:3), fractionated with chloroform, ethyl acetate, saturated n-butanol and the residue fraction to get corresponding fractions, 10g, 3g, 7.4g, and 10.0g respectively. All four fractions were submitted for biological activity studies and found chloroform fraction showed melanin promotion and elastase inhibition activities. The chloroform fraction was purified by using Vacuum Liquid Chromatography technique by using chloroform, chloroform: ethyl acetate mixture (9:1, 8:2 and 1:1). A total of 10 fractions were collected (250 ml each) were collected and the fractions were analyzed by thin layer chromatography and fractions showing similar TLC behavior were combined to obtain three major fractions, Fr. 1 (2.56 g), Fr. 2 (1.85 g) and Fr.3 (2.3 g). All fractions were submitted for biological activity and fraction.2 showed more potent activity. Fraction 2 was further purified by another small column of silica gel using chloroform as an eluent to obtain pale green colored solid (750 mg) which was further re-crystallized with chloroform and acetone to get white amorphous powder, oleanolic acid (**1**, 520mg).[17] Fraction 3 which was purified by another small column using mixture of chloroform : ethyl acetate (1:1) yielded colorless solid, 4-hydroxy-benzaldehyde (**2**, 145mg).[18] The fraction 1 did not yield any pure compound. Compound **1** has been submitted for biological studies and showed good elastase inhibition. The ethyl acetate fraction showed one major spot along with some pigments. It was further purified by another small column using chloroform: ethyl acetate (1:1, 2:8) and ethyl acetate. A total of 30 fractions (each 50 ml) were collected, combined homogeneous fractions and concentrated to get three sub fractions, EAF1(0.42g), EAF2 (0.9g) and EAF3 (1.2 g) respectively. The fraction, EAF2 showed solid nature and only one major spot on TLC. The fraction was crystallized with methanol to get colorless solid, stigmasterol-3-O-β-D-glucopyranoside (**3**, 175 mg).[19] The butanolic fraction was found to be hazy, crystallized with methanol to yield one crystalline compound, D-quercitol (**4**, 830 mg)[6] which showed melanin promotion activity (88% at 20 µg/ml) and elastase inhibition activity (6.2%@ 40ug/ml).

RESULTS AND DISCUSSION

Compound 1 (Oleanolic acid): Amorphous powder, mp: 244-46^{o}C, IR (υ cm^{-1}): 3448, 1691, 1458, 1031, 758; ^{1}H NMR (d_4-MeOH, 400MHz) : δ 0.77 (3H, s), 0.84 (3H, s), 0.87 (3H, d, J=6.4Hz), 0.94 (3H, d, J=6.4Hz), 0.95 (3H, s), 0.97 (3H, s), 1.11 (3H, s), 3.15 (1H, dd, J=4.2, 6.1Hz), 5.21 (1H, t, J=3.3 Hz). ^{13}C NMR (D_4-MeOH, 100MHz) : δ 14.5, 14.9, 16.1, 16.3, 18.0, 20.1, 22.6, 26.4, 27.3, 27.8, 30.2, 33.8, 36.6, 38.3, 38.4, 38.9, 39.3, 41.7, 52.9, 55.2, 78.2, 125.4, 138.1, 180.1; EIMS (rel. int): m/z 456 (M^{+}, 2%), 248 (100%), 203 (43%), 189 (10%), 133 (40%).

Compound 2 (4-Hydroxybenzaldehyde): Colorless solid, mp:113^{o}C, ^{1}H NMR (d_6-acetone, 400 MHz): δ 7.0 (2H, d, J=8.6Hz), 7.81 (2H, d, J=8.6Hz), 9.8 (1H, s); ^{13}C NMR (d_6-acetone, 100 MHz): δ 190.0, 162.9, 131.8, 131.8, 129.4 115.6, 115.6; EIMS (rel. int): m/z 122 (M^{+}, 45%), 93 (100%), 77 (56%).

Compound 3 (Stigmasterol-3-O-β-D-glucopyranoside): (d_6-DMSO, 400 MHz): δ 0.52 (3H, s), 0.75 (6H, s), 0.78 (6H, d, J=6.4Hz), 1.00 (3H, d, J=6.4 Hz), 2.90 (1H, m), 3.03 (2H, m), 3.12 (1H, m), 3.42 (1H, m), 3.53 (1H, m), 3.63 (1H, dd), 4.21 (1H, d), 4.18 (1H, t), 4.70 (2H, d), 5.07 (1H, m), 5.16 (1H, dd).

Compound **4** (**D-Quercitol**): Colorless crystals, mp: 233-35^{o}C; ^{1}H NMR (D_2O, 400 MHz): δ 1.69 (1H, ddt, J= 3.0, 0.7, 13.0Hz), 1.88 (1H, dt, J=2.6, 10.3Hz), 3.43 (1H, t, J=9.4Hz), 3.60 (1H, m), 3.80 (1H, t, J=3.1Hz), 3.88 (1H, t, J=3.1Hz); ^{13}C NMR (D_2O, 100 MHz): δ 33.1, 68.4, 68.7, 70.8, 72.1, 74.4; EIMS (rel. int): m/z 164 (M^+, 33%), 146 (91%), 129 (54%).

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 (the 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 compounds were tested and results were mentioned in the table 1.

Melanin promotion activity: The melanin promotion activity of crude extract and isolated compounds and lupeol (control) were studied in cell lines (B16F10 melanoma cells).[10] The assay method is most precise and reliable. The crude methanolic extract showed potent activity and one of the isolated compound, **4** showed moderate activity and the results were mentioned in the table 1.

Table 1: Comparative data of biological activity

Sl. No.	Extract/ Fraction / Compound	Elastase Inhibition (µg/ml)	Melanin promotion (µg/ml)
1	Control	IC_{50}=13.1 (Ursolic acid)	401% at 15 (Lupeol)
2	Methanolic extract	30.9% at 40	140% at 50
3	Compound **1**	IC_{50}=16.0	Not active
4	Compound **3**	5.6% at 40	Not active
5	Compound **4**	6.2% at 40	88% at 20

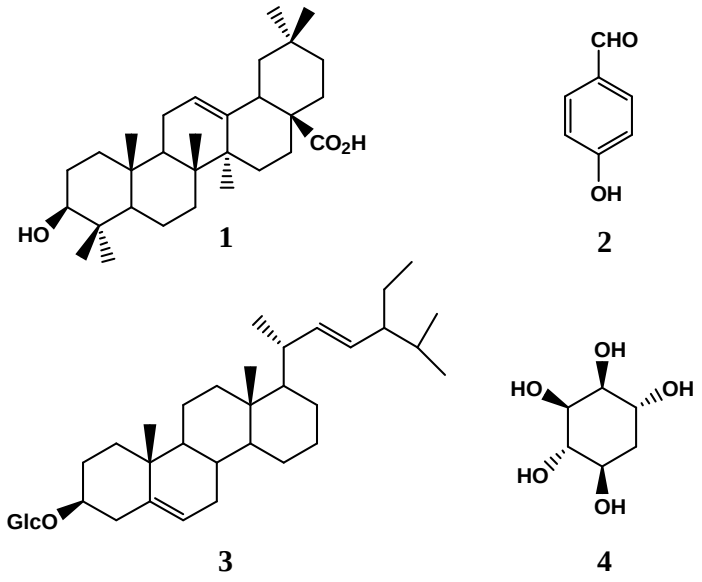

Figure 1: Chemical constituents from *Mimusops elengi*

CONCLUSION

The compound D-quercitol (**4**) is common constituent in all parts of the plant, where as oleanolic acid (**1**) and stigmasterol-3-O-β-D-glucopyranoside (**3**) were previously reported from this plant. The compound, 4-hydroxybenzaldehyde (**2**) is the first report from this plant. The biological studies, melanin promotion and elastase inhibition are also first time for this plant.

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