Short Communicaton

Phytochemical evaluation of the leaves of *Aegle marmeloes* L. (L.) – an important medicinal plant

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Abstract

Aegle marmeloes L. (L.), belonging to Rutaceae family, is a widely used medicinal plant. This plant is known to possess hypoglycemic, antimicrobial, anti-inflammatory, radio protective, anti-oxidative and anticancer activities. The present study is aimed to find out the important phytochemicals present in *Aegle marmeloes* L. (L.) leaves using FTIR and GC-MS. Most structurally similar compound as per FTIR was L (-)-glyceraldehyde unnatural form. Phytol was detected as a major ingredient in GC-MS analysis.

Keywords: *Aegle marmeloes* leaves, FTIR, GCMS, Rutaceae

Aegle marmeloes L. (L.) is a commonly used medicinal plant in Ayurveda. It belongs to the genus Sapindales and family Rutaceae. *A. marmeloes* is the only member of the monotypic genus *Aegle.* This deciduous shrub or small to medium-sized tree is a native of Southeast Asia and India. It is commonly known by names like bael, bengal quince, golden apple and japanese bitter orange. The plant possesses hypoglycemic, antimicrobial, anti-inflammatory, anti-oxidative, anticancer, radio protective (Yadav and Chanotia, 2009) and hepatoprotective (Jayachandra and Sivaraman, 2011) activities. According to National Medicinal Plants Board, Govt. of India (Kala et al., 2006), it is a prioritised medicinal plant. The identification and standardisation of active ingredients of this plant is essential for the integration and utilisation of this herb scientifically in neutraceuticals and ayurvedic preparations. Hence the present study was initiated to find out the active ingredients present in locally available *A. marmeloes* leaves.

A. marmeloes L. (L.). mature leaves were collected during the month of February 2018 from Mannuthy area of Thrissur district and authenticated at Department of Botany, St Thomas College, Thrissur (Plate 1). Duly authenticated herbarium was kept in the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy. The leaves were shade dried and pulverized using an electrical pulveriser. For GC-MS analysis fifty grams were taken and extracted with methanol using accelerated solvent extractor (Thermoscientific, model: Di1x ASE 150). The methanolic extract was then concentrated using a rotary vacuum evaporator under reduced pressure and temperature and stored under refrigeration (4ºC) until further use.

Functional groups present in *A. marmeloes* leaf powder were identified using Fourier transform infrared (FTIR) spectroscopy (Perkin Elmer, FTIR spectrophotometer, Singapore). Two mg of *A. marmeloes* leaf powder and 298 mg of dry fine

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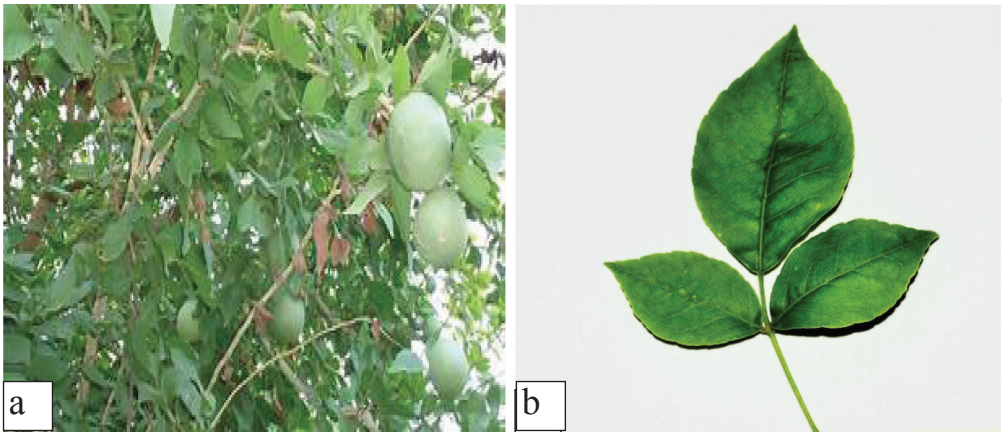

Plate 1. *Aegle marmeloes* -– a. Whole plant, b. mature leaves.

powder of potassium bromide (KBr) were weighed and transferred into a mortar and mixed well. The KBr-sample mixture was transferred to an evacuable die that had a barrel diameter of 13 mm and the die was pressed at around 8 to 10 tons for 1 to 2 minutes in a hydraulic hand press. Re-crystallization of the KBr resulted in a clear transparent disk about 1 mm thick and the infrared spectrum was recorded in the scan range of 4000 cm^{-1} to 400 cm^{-1}on FTIR spectrophotometer with a resolution of 0.5 cm^{-1}. The structurally related compounds were identified through Fluka library supplied by Perkin-Elmer (Swapna et al., 2012).

The GC-MS analysis of the methanolic extract was carried out at Kerala Forest Research Institute, Peechi, Thrissur using Shimadzu GCMS (Model Number: QP2010S). The compounds were separated on Rxi-5Sil MS capillary column (30 m × 0.25 mm; I.D. 0.25 ìm film). The sample, dissolved in methanol and filtered through 0.22ì syringe filter, was used for analysis. The column oven temperature was programmed from an initial temperature of 80ºC (4 min), then temperature raised to 280ºC at the rate of 5ºC min^{-1}, finally 280ºC was maintained isothermally with a final time of 6 min. The injection temperature and ion source temperature were 260 and 200ºC, respectively. Helium (99.999%) was used as the carrier gas with a flow rate of 1 ml min^{-1}. The ionizing energy was 70 eV. All the data were obtained by collecting the full-scan mass spectra within the scan range 50–500 amu. Compounds were identified using the National Institute of Standards and Technology (NIST 11) and Wiley 8 library (Victoria et al., 2014).

Based on the search score, most structurally similar compound yielded from leaf powder of *Aegle marmeloes* was L (-) - glyceraldehyde unnatural form. The other structurally similar compounds

Table 1. The result of FTIR analysis of *Aegle marmeloes* leaf powder

Search Score	Structurally related compound	Type of compound
0.637221	L (-)-glyceraldehyde unnatural form	Aldehyde
0.529118	Tomatine	Glycoalkaloid
0.523804	Taurocholic acid sodium salt	Bile conjugate
0.495462	Chitin	Carbohydrate
0.495127	Octyl-beta-d-glucopyranoside	Protein
0.470864	Heptyl-beta-d-glucopyranoside	Protein
0.469305	2-(methylthio)-ethylamine	Amine
0.463993	Digitonin	Steroid glycoside
0.440996	Ethylene glycol polymer-bound	Alcohol
0.43215	Dodecyl-b-d-glucopyranoside	Protein

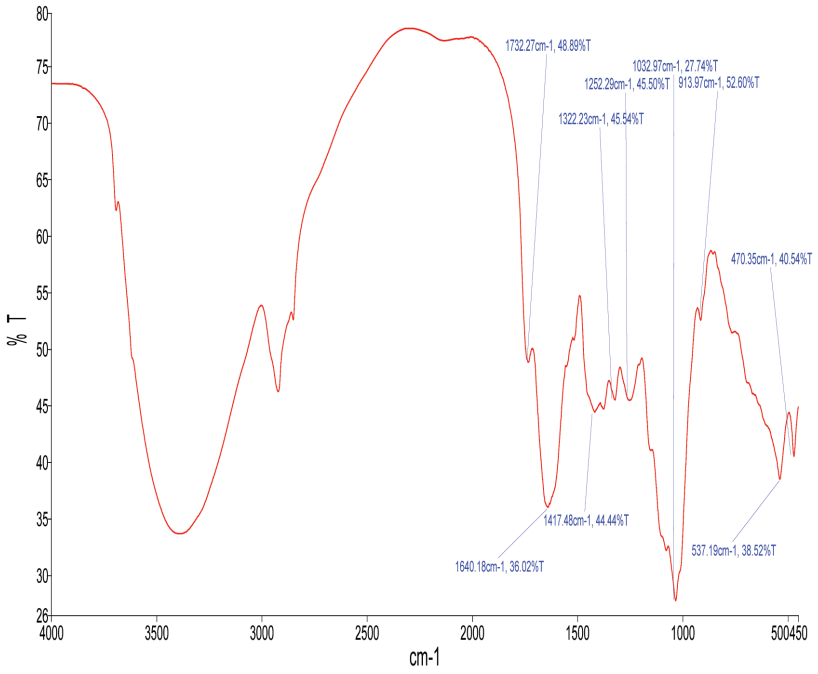

Figure 1. Peak obtained on FTIR analysis

identified were tomatine, taurocholic acid sodium salt, chitin, octyl-beta-d-glucopyranoside, heptyl-beta-d-glucopyranoside, 2-(methylthio)-ethylamine, digitonin, ethylene glycol polymer-bound and dodecyl-b-d-glucopyranoside (Table 1, Figure 1). Ariharan et al. (2015) confirmed the presence of alkyl halides, alkynes, aromatics, alkenes, aromatics, esters, alcohols, amines, alkanes, nitro compounds and amide compounds. Less number of functional groups could be detected during the present study.

On GC-MS analysis, about 10 active principles were found in the methanolic extract of *Aegle marmeloes* L. (L.) leaves. A wide range of alcohols, fatty acids, esters, diterpenoids, triterpenoids and many other acyclic alkanes were found in the extract. .alpha.-curcumene, (+)- (9.56%), .alpha.-zingiberene (14.41%), Mycrene (1.22%), .beta.-sesquiphellandrene (12.85%), ethanone, 1,2-dicyclopropyl- (0.90%), 1,7-nonadiene, 4,8-dimethyl-1-nitro-, (e)- (2.25%), citronellyl propionate (4.39%), nonanoic acid, methyl ester (2.23%), phytol (50.88%) and 4,8-dimethyl-3(e),7-

Table 2. The results of GC-MS analysis of methanolic extract of *Aegle marmeloes* leaves

Peak	Retention time (s)	Area (%)	Name	Base (m/z)
1	18.601	9.56	.Alpha.-curcumene, (+)-	132.15
2	18.950	14.41	.Alpha.-zingiberene	119.10
3	19.271	1.22	Mycrene	69.10
4	19.677	12.85	.Beta.-sesquiphellandrene	69.10
5	23.725	0.90	Ethanone, 1,2-dicyclopropyl-	69.05
6	26.222	2.25	1,7-nonadiene, 4,8-dimethyl-1-nitro-, (e)-	69.05
7	26.689	4.39	Citronellyl propionate	68.05
8	28.494	2.23	Nonanoic acid, methyl ester	74.05
9	32.080	50.88	Phytol	71.05
10	43.259	1.31	4,8-dimethyl-3(e),7-nonadienyl thioacetate	69.05

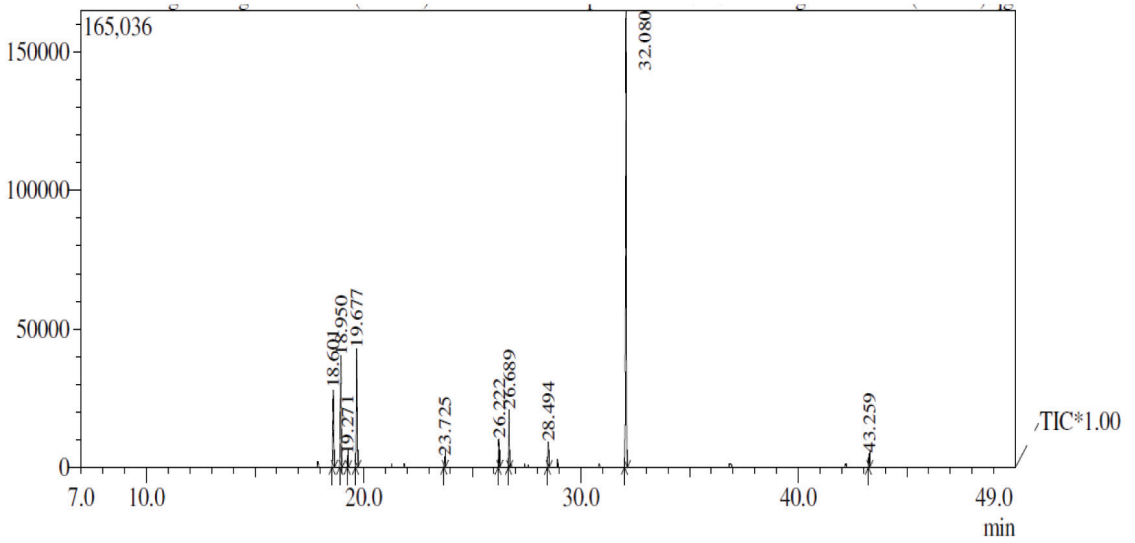

Figure 2. Chromatogram obtained from GC-MS analysis of methanolic extract of *Aegle marmeloes* leaves

nonadienyl thioacetate (1.39%) (Table 2, Fig 2) were the active principles identified. Phytol was the major characterised component followed by beta-sesquiphellandrene and alpha-zingiberen as other main components.

Alpha-curcumene is an aromatic monoterpenoid and known to have cytotoxic and antiviral properties and belongs to the family of sesquiterpenes. Alpha.-zingiberene is a cycloalkene compound with the ability to inhibit generation of amyloid-beta proteins and their aggregates. Mycrene is a monoterpene with anti-inflammatory, anti-nociceptive and muscle relaxant properties. Sesquiphellandrene is known to have antifungal and insecticidal properties. Phytol is an antimicrobial, anticancer, anti-inflammatory and diuretic agent (Kumar et al., 2010). It is a key acyclic diterpene alcohol that is a precursor for vitamins E and K1. It is also having carminative and antiviral properties (Olofsson et al., 2014). Shubhangi and Rupali (2017) reported presence of 15 compounds in the methanolic extract of *A. marmeloes* namely 13- Heptadecyn-1-ol, Dibutyl phthalate, 1,2- Benzenedicarboxylic acid, Phthalic acid, Phytol, Ethyl iso- allocholate, Diisooctyl phthalate, Vinyl phenyl acetonitrile,13-Docosenamide, Squalene, Pentoic acid, Oxirane, Vitamin E, á- Tocopherol, Carotene, 9,12,15-Octadecatrienoic acid, ç- Sitosterol. Presence of mycrene, phellandrene and phytol in *A. marmeloes* was also observed by Verma et al. (2010). Mujeeb et al. (2014) detected phytol as one of the components of *A. marmeloes* leaves. Yadav et al. (2013) demonstrated presence of alpha zingiberene, mycrene and curcumene in the oil obtained from *A. marmeloes* leaves. Bioactive compounds like alpha.-curcumene, alpha.-zingiberene, beta.-sesquiphellandrene and phytol have health benefits and are capable as nutraceuticals.

The differences in the compounds reported in this study and previous results might be due to difference in the geographical locations of plants as well as the nutrient content of the soil which might influence the growth and chemical composition of the plants. Thus the present study has confirmed the presence of important phytochemicals which render therapeutic properties to this medicinal plant.

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