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Phytochemical analysis of *Aegle marmelos* fruit extract by gas chromatography-mass spectroscopy

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Abstract
Aegle marmelos (bael), a fruit with immense nutritional value had been a part of the Indian and traditional system of medicine for a very long time. The fruits possess rich medicinal benefits due to the presence of several phytochemicals, yet its usage is negligible and underexploited. The bioactives present in the fruits are extracted, isolated, identified and characterized in order to arrive at a clear understanding of the bioactivities. The current study takes into consideration the GC-MS technique for the identification, simultaneous quantification, and analysis of volatile compounds. GC-MS analysis revealed the presence of six major compounds *viz.*, Heraclenin, Imperatorin, Methoxsalen, Germacrene B, Alpha-Guaiene, and Caryophyllene. These compounds exhibit varied bioactivities such as anticancer, antioxidant, anti-inflammatory, antibacterial, antifungal, antiviral and neuroprotective properties. Thus bioactives are important in nutraceutical and pharmacological industries but their bioavailability is a major limitation. Hence, future researches should primarily focus on developing novel techniques to enhance their bioavailability of bioactive compounds.

Keywords: *Aegle marmelos*, extraction, bioactive, GC-MS, bioactivity

Introduction
Various undervalued fruits had gained prominence in recent years due to their bioactive constituents with excellent therapeutic potential. Among them *Aegle marmelos* (L.) Correa, commonly known by the name Bael, is a fruit tree belonging to the Rutaceae family which is indigenous to India and was considered to be inevitable in the Indian Medicine system due to its ethnomedical properties. Almost every part of the tree which includes root, bark, fruit, leaf, and flower was used in the traditional system of medicine and Ayurveda to treat many ailments (Baliga *et al.*, 2011) [2]. The fruit pulp is rich in fiber, water, iron, sugar, fat, protein, vitamins (A, B, C and riboflavin), phosphorus, potassium, calcium and micronutrients .In addition to that Vitamin C is responsible for antioxidant properties and the presence of substantial amount of flavonoid and phenolic contents is significant in treating many diseases (Biswas *et al.*, 2023) [3]. The fruit extract exhibits various pharmacological activities which includes antidiarrheal, antioxidant, antidiabetic, antimicrobial, anti-proliferative, hepatoprotective, anticancer, antipyretic, and antiulcer properties (Sharma *et al.*, 2022) [16]. The various phytochemical identified from the fruit extract includes carotenoids, phenolics, alkaloids, pectins, tannins, coumarins, and flavonoids (Manandhar *et al.*, 2018) [11]. The presence of coumarins such as sesquiterpenic coumarin ethers, diterpenic coumarin ethers, triterpenic coumarin ethers, auraptene, epoxyauraptene, marmelosin, imperatorin, alloimperotorin, scoparone, scopoletin, umbelliferone and marmin were reported in *A. marmelos* (Venthodika *et al.*, 2021) [19]. Of these coumarins isolated, heraclenin and imperatorin have become areas of interest for many researchers due to their bioactivity which will play an immense role in the medical field and further research in drug modeling. Though the cultivation is easy, fruit with excellent nutritional value have been underutilized and underexploited. Owing to its high nutritional value and the presence of various phytochemicals, the processing of fruits into various products is gaining importance and has become a major focus area of research (Murthy *et al.*, 2020) [12].

Materials and Methods
Chemicals and Solvent
Ethyl acetate (AR grade) was purchased from Avra, India, and Hexane (AR grade) from Chempure, and Methanol (HPLC grade) from CDH Gujarat, India.

Collection of Fruits

Fresh unripe bael fruits were harvested as a single source for compound extraction from a nine-year-old bael tree in P.N. Pudur (latitude 11.0202 and longitude 76.9211) of Coimbatore, Tamil Nadu, India during the fruiting season of May 2023.

Extraction of Bael extract

Fresh unripe fruits were cut into equal half then seeds and hulls were removed from the mesocarp portion. The cleaned mesocarp pulp portion was chopped into fine pieces and shade dried until complete removal of moisture. The dried mesocarp portion was blended and sieved through Mesh No 70. Blended mesocarp powder 50g was added into 100 ml ethyl acetate in the ratio of (1:2) and kept in an orbital shaker @ 100 rpm for 24 hours then filtered through muslin cloth followed by Whatman filter paper 40. The extract was concentrated in a rotary evaporator under reduced pressure at 42 degrees Celsius.

GC-MS Profiling of *Aegle marmelos* (L.)

The GC-MS analysis of *A.marmelos* was carried out on Clarus SQ 8c Gas Chromatography-Mass spectrometer from Perkin Elmer. The Instrument program settings were injector port temperature 220 °C, Interface temperature 250 °C, Injector was used in split less mode. The DB-5 standard non-polar column (0.25 mm OD ×0.25 µm ID × 30 Meter length M/s Agilent Co., USA) with Helium as the carrier gas at 1ml/min was applied. The MS was programmed to scan at 50 to 550 Da. The instrument has inbuilt libraries NIST MS Search 2.2 v- Containing more than five references for searching and matching the spectrum.

Results and Discussion

GC-MS Chromatogram spectra obtained from *A. marmelos* showed a total of 40 peaks (Figure: 1) and on the basis of peak area percentage more significant than 1% and the molecular weight, the major six bioactive compounds are listed in Table 1. And their individual GC-MS Spectral analysis follows from (Figure 2- Figure 6).

The major compounds and their bioactivity reported so far includes, Heraclenin is known to exhibit anti-inflammatory, anticoagulant, cytotoxic, antiplatelet, mild phototoxic and photo-mutagenic activities (Venthodika *et al.*, 2021) [19]. It is also observed that heraclenin has the ability to improve osteoblast differentiation, mineralisation, and bone regeneration (Shanmugam *et al.*, 2019) [15]. Antifungal action of heraclenin against *Xanthomonas oryzae* and *Colletotrichum lindemuthianum* were also reported (Santhana Krishnan *et al.*, 2016) [14]. A study investigated the pharmacological activities of imperatorin enlisted the following activities which includes anticancer, neuroprotection, anti-inflammatory, anti-hypertension, and antibacterial (Deng *et al.*, 2020) [4]. Protective effects of imperatorin on the central nervous system and cardiovascular system, anticancer and antiviral effects were also noticed (Nasser *et al.*, 2019) [13]. Methoxsalen was observed to have role in photochemotherapy for treatment of epidermal diseases like psoriasis and vitiligo (Maitray and Rishi 2017) [10]. Methoxsalen Plays an important role in preventing diabetes induced osteoporosis (Ham *et al.*, 2019) [8]. Germacrene B, a sesquiterpene was found to exhibit antimicrobial activities including antibacterial and antifungal activities (Badalamenti *et al.*, 2022) [1]. In addition, antiproliferative activity was also observed (Siqueira *et al*., 2015) [17]. Alpha guaiene was found to have antibacterial and anticancer properties (Zhang *et al.*, 2017) [20]. Anti-inflammatory, antibacterial, osteoporosis, steatohepatitis, anticonvulsant, myorelaxant, sedative and antidepressive properties of caryophyllene were reported (Francomano *et al.*, 2019) [7]. Caryophyllene, a sesquiterpene exhibited biological activities such as antioxidant, antimicrobial antifeedant, phytotoxic and acaricidal activities. Antifungal activity is noted for caryophyllene (Rambo *et al.*, 2022) [18]. Anticancer effect of Caryophyllene by inhibiting the inflammatory factors expression was reported by various studies (De Lima *et al.*, 2014; Fidyt *et al.*, 2016) [5, 6].

Table 1: Major bioactive found in *Aegle marmelos* fruit extract

Peaks	RT(Min)	Area %	Compound name	Molecular formula	Molecular weight
40	29.66	68.597	Heraclenin	$C_{16}H_{14}O_5$	286.28
38	28.26	6.871	Imperatorin	$C_{16}H_{14}O_4$	270.28
34	22.74	3.863	Methoxsalen	$C_{12}H_8O_4$	216.19
18	13.24	2.23	Germacrene B	$C_{15}H_{24}$	204.35
8	11.977	2.07	Alpha-Guaiene	$C_{15}H_{24}$	204.35
4	10.55	1.647	Caryophyllene	$C_{15}H_{24}$	204.35

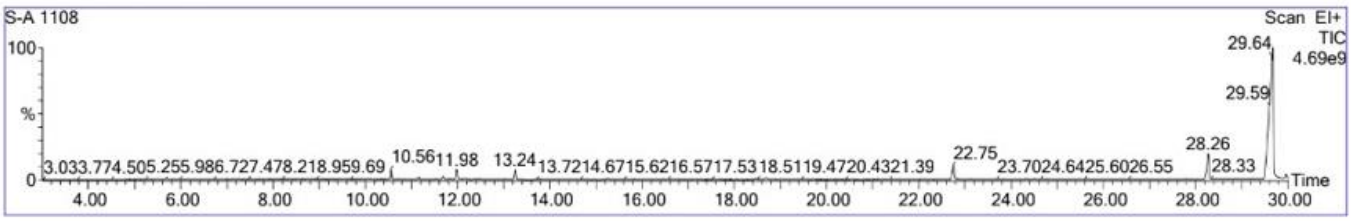

Fig 1: GC-MS Spectral analysis of ethyl acetate extract of *Aegle marmelos* fruit

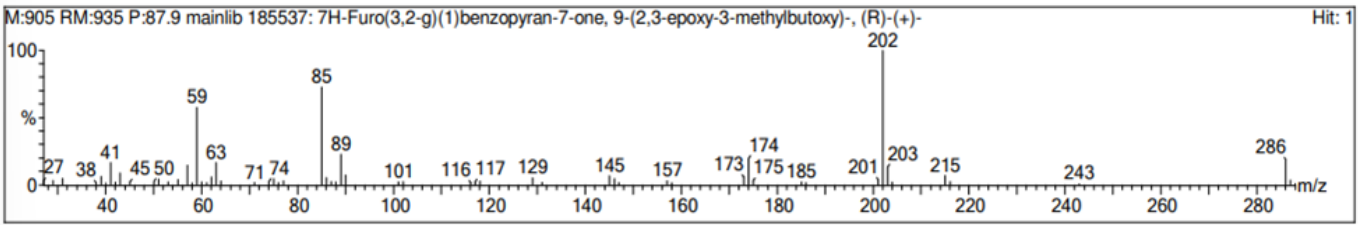

Fig 2: GC-MS chromatogram of Heraclenin

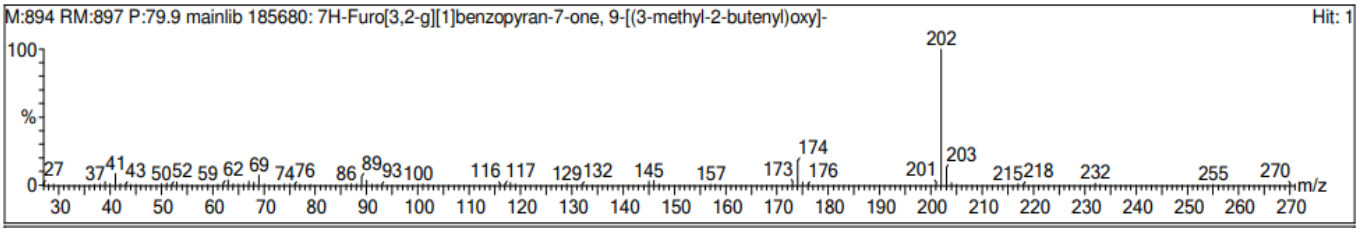

Fig 3: GC-MS chromatogram of Imperatorin

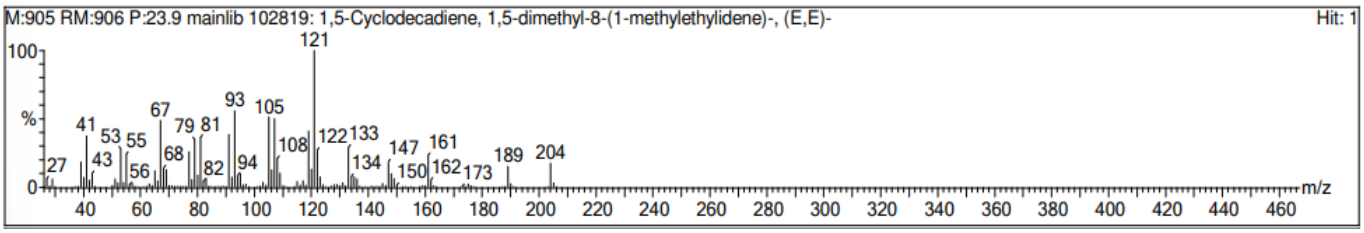

Fig 4: GC-MS chromatogram of Methoxsalen

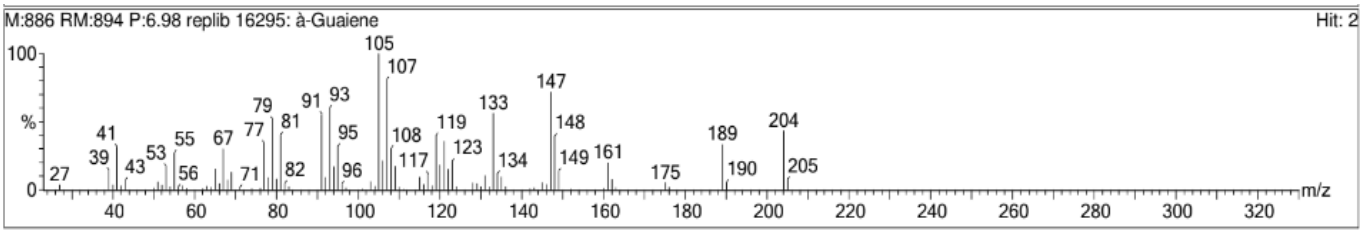

Fig 5: GC-MS chromatogram of Alpha-Guaiene

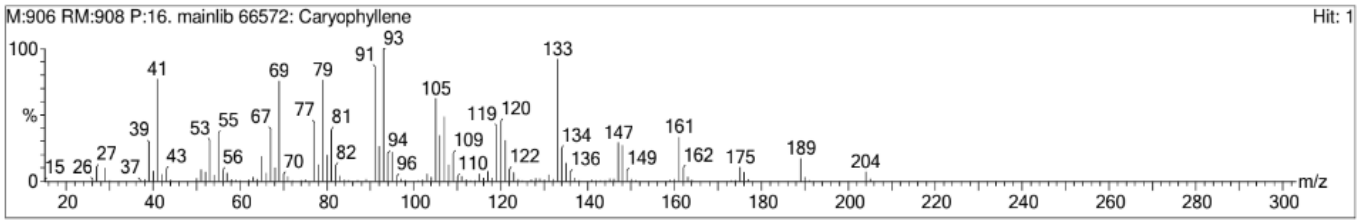

Fig 6: GC-MS chromatogram of Caryophyllene

Conclusion

The therapeutic properties of *A. marmelos* fruit is evident from the bioactives present in it. The data obtained from the present study for analyzing the bioactives using GC-MS method confirmed the presence of 6 major bioactive compounds Heraclenin, Imperatorin, Methoxsalen, Germacrene B, Alpha-Guaiene and Caryophyllene in the fruit pulp. The presence of bioactive compounds could exert significant roles in pharmacological studies. In depth knowledge on the reported bioactive compounds will be facilitated by exploring the bioactivities associated with them. However the poor bioavilability of herbal extracts curtail their potential in pharmacological and clinical studies. In order to completely exploit the therapeutic benefits of the bioactives, future studies should extensively focus on developing novel methods to enhance the bioavalability of these compounds.

References

1. Badalamenti N, Bruno M, Formisano C, Rigano D. Effect of Germacrene-Rich Essential Oil of *Parentucellia latifolia* (L.) Caruel Collected in Central Sicily on the Growth of Microorganisms Inhabiting Historical Textiles. Natural Product Communications. 2022;17(4). 1934578X221096963.
2. Baliga MS, Bhat HP, Joseph N, Fazal F. Phytochemistry and medicinal uses of the bael fruit (*Aegle marmelos* Correa): A concise review. Food Research International. 2011;44(7):1768-1775.

3. Biswas SK, Abedin MZ, Dey BC, Al Reza MS, Bari L, Zubair MA. Nutritional Composition and Bioactive Compounds of Bael (*Aegle marmelos*) and Development of Functional Food Products. Food and Nutrition Sciences. 2023;14(4):328-340.
4. Deng M, Xie L, Zhong L, Liao Y, Liu L, Li X. Imperatorin: A review of its pharmacology, toxicity and pharmacokinetics. European Journal of Pharmacology. 2020;879:173124.
5. De Lima VT, Vieira MC, Kassuya CAL, Cardoso CAL, Alves JM, Foglio MA, *et al*. Chemical composition and free radical-scavenging, anticancer and anti-inflammatory activities of the essential oil from *Ocimum kilimandscharicum*. Phytomedicine. 2014;21(11):1298-1302.
6. Fidyt K, Fiedorowicz A, Strządała L, Szumny A. β-caryophyllene and β-caryophyllene oxide—natural compounds of anticancer and analgesic properties. Cancer medicine. 2016;5(10):3007-3017.
7. Francomano F, Caruso A, Barbarossa A, Fazio A, La Torre C, Ceramella J, *et al*. β-Caryophyllene: A sesquiterpene with countless biological properties. Applied Sciences. 2019;9(24):5420.
8. Ham JR, Choi RY, Lee HI, Lee MK. Methoxsalen and bergapten prevent diabetes-induced osteoporosis by the suppression of osteoclastogenic gene expression in mice. International Journal of Molecular Sciences. 2019;20(6):1298.
9. Kasaian J, Alesheikh P, Mohammadi A. Chemical compositions and biological activities of *Scutellaria* genus essential oils (Lamiaceae). Jundishapur Journal of Natural Pharmaceutical Products. 2020;15(3).
10. Maitray A, Rishi P. Methoxsalen-induced macular toxicity. Indian Journal of Ophthalmology. 2017;65(11):1243.
11. Manandhar B, Paudel KR, Sharma B, Karki R. Phytochemical profile and pharmacological activity of *Aegle marmelos* Linn. Journal of integrative medicine. 2018;16(3):153-163.
12. Murthy HN, Bhat MA, Dalawai D. Bioactive compounds of bael (*Aegle marmelos* (L.) correa). Bioactive compounds in underutilized fruits and nuts; c2020. p. 459-486.
13. Nasser MI, Zhu S, Hu H, Huang H, Guo M, Zhu P. Effects of imperatorin in the cardiovascular system and cancer. Biomedicine & Pharmacotherapy. 2019;120:109401.
14. Santhana P, Krishnan V, Karthikeyan G, Janaki P, Poonkodi B, Sathya R. Isolation of *Heraclenin* from *Aegle marmelos* correa and Screening for its Antimicrobial Activity Through *in vitro* & in silico Studies. The Natural Products Journal. 2016;6(2):134-141.
15. Shanmugam H, Dharun VN, Biswal BK, Chandran SV, Vairamani M, Selvamurugan N. Osteogenic stimulatory effect of heraclenin purified from bael in mouse mesenchymal stem cells *in vitro*. Chemico-Biological Interactions. 2019;310:108750.
16. Sharma N, Radha Kumar M, Zhang B, Kumari N, Singh D, Lorenzo JM. *Aegle marmelos* (L.) Correa: an underutilized fruit with high nutraceutical values: a review. International Journal of Molecular Sciences. 2022;23(18):10889.
17. Siqueira CAT, Serain AF, Pascoal ACRF, Andreazza NL, de Lourenço CC, Góis Ruiz ALT, *et al.* Bioactivity and chemical composition of the essential oil from the leaves of *Guatteria australis* A. St.-Hil. Natural product research. 2015;29(20):1966-1969
18. Rambo MA, Soares KD, Danielli LJ, Lana DFD, Bordignon SADL, Fuentefria AM, *et al*. Biological activities of essential oils from six genotypes of four Ocotea species. Brazilian Journal of Pharmaceutical Sciences. 2022;58:e181097.
19. Venthodika A, Chhikara N, Mann S, Garg MK, Sofi SA, Panghal A. Bioactive compounds of *Aegle marmelos* L., medicinal values and its food applications: A critical review. Phytotherapy Research. 2021;35(4):1887-1907.
20. Zhang HY, Gao Y, Lai PX. Chemical composition, antioxidant, antimicrobial and cytotoxic activities of essential oil from Premnamicrophylla Turczaninow. Molecules. 2017;22(3):381.