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Isolation and Characterization of Phytoconstituents from Some Medicinal Plants of Rutaceae Family

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ABSTRACT:
Natural metabolites from medicinal plants, either as pure compounds or as standardized extracts, provide number of opportunities for new drug leads because of the unmatched availability of chemical diversity. Due to an increasing demand for chemical diversity in screening programs, seeking therapeutic drugs from natural products, interest particularly in edible plants has grown throughout the world. The plants from Rutaceae family as mentioned above *Feronia limonia* Linn. and *Aegel marmelos* Linn. containing phytoconstituents generate area to evaluate them for antiepileptic, antioxidant and antimicrobial effects. The literature updates enlist variety of phytochemicals as phenolics, alkaloids, flavonoids, glycosides, steroids; tannins etc. still some category of phytoconstituents need to identify and screen for antiepileptic, anti-inflammatory, antimicrobial potential. So, these investigations were aimed to pinpoint the active components besides finding out new structural leads for future led drugs in treatment of epileptic conditions. Isolated ethanolic fractions of both plants collected and characterization performed through spectral techniques like IR, ^{1}H NMR, ^{13}C NMR**,** Mass spectrum. The structural elucidation of the lead bioactive fractions confirms the presence of two potent flavonoid derivatives, which validates the significance of antimicrobial and antioxidant activities. The identification of these specific chromen-1-ium (flavylium) derivatives provides a definitive chemical basis for the therapeutic potential of Feronia limonia and Aegle marmelos.

MATERIAL AND METHODS
Collection and Authentication of the Plant material:
Feronia limonia Linn. and Aegel marmelos Linn. plant leaves were collected from Ahilyanagar District of Maharashtra, India. The plant specimen was authenticated by Dr. D.L.Shirodkar Botanist, Botanical Survey of India, Pune.

Extraction of Plant Material:
Feronia limonia Linn. and Aegel marmelos Linn. plant leaves were collected and successively extracted by

continuous Soxhlet extraction method separately with a following range of polarity solvents, i.e., nonpolar to polar solvents. Each powered batch was loaded for extraction. The five solvents, viz. Hexane, chloroform, ethyl acetate, ethanol and water were used for extraction. Then the resultant extract was filtered and the marc was separated. The filtrate was evaporated to dryness on a hot plate at 45°C to get a semi solid extract.

Column Chromatography of Extracts:
The stationary phase was prepared with activated silica for column chromatography and mobile phase n-Hexane. Clean glass column and fixed stationary phase Adsorbent was allowed to settle and test samples were prepared by mixing the extract with activated silica until it became free flowing. Mobile phase selected proportion was added to the column. All precautions were taken during laboratory procedures The column fractions were eluted with mobile phase in gradient manner. Collected *Feronia limonia* ethanol fraction (FLE-B3) and *Aegel marmelos* ethanol (ALE-D4) fraction were concentrated and stored at room temperature All collected fractions were mark separately and concentrated. Isolated fraction may separate the complex phytochemicals which will potentiate further pharmacological activities.

Structural characterization of bioactive fractions
Thus, it was felt necessary to elucidate bioactive fractions for determination of probable structure by spectroscopic analysis. So fractions were screened for Fourier Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance (^{1}H NMR, ^{13}C NMR) and Mass Spectroscopy. Separated fraction details and code assigned as follows table no. 1.

Table No.1: Details of test compound and number assigned in structural analysis

Test compound Number	Test Fraction Code	Details of Source
I.	**FLE-B3**	*Feronia limonia* ethanol fraction B3
II.	**ALE-D4**	*Aegel marmelos* ethanol fraction D4

1. Infrared spectroscopy studies:
Infrared absorption spectra of the test compounds were recorded on Shimadzu 8400-S Fourier Transform Infrared (FTIR) Spectrophotometer using Potassium Bromide (V max in cm-1).

2. Nuclear magnetic resonance (NMR) studies:
It counts the number and environment of proton and fragmented carbon ions in the chemical structure in the form of peaks in ^{1}H NMR and ^{13}C NMR. The ^{1}H NMR and ^{13}C NMR spectra were recorded using NMR Bruker Advance-II 400 MHz spectrometer respectively and chemical shifts are given in units as ppm, downfield from Tetra Methyl Silane (TMS) as an internal standard at Cryogen NMR services, Mumbai.

3. Mass Spectroscopy:
Mass Spectroscopy (MS) is an analytical technique for the determination of the elemental composition of isolated test molecules. Mass spectra were recorded on the ESI-MS Mass spectrometer at Cryogen MASS services, Mumbai.

Details of test compound observations and results for structural elucidation as follows,

Compound I details:

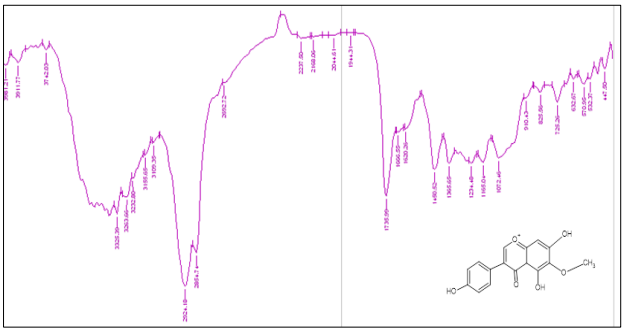

Figure No. 1: IR spectrum of compound 1

IR (cm^{-1}) KBr: 3394.83 (O-H stretching), 2924.18 (C-H stretching), 3285.95(HC=CH stretching), 1736.50 (C=O Stretching),1658.84(C=C), 1458.23(CH from CH2), 1373 [C-H stretching (CH2 (CH3)2)], 1157.33(OH-bending) 1072.46 (C-C stretch)

Chinese Journal of Health Management
Volume 20 Issue 5, Year of Publication 2026, Page 1164-1169 | DoI-10.564220/1674-0815.2026.140

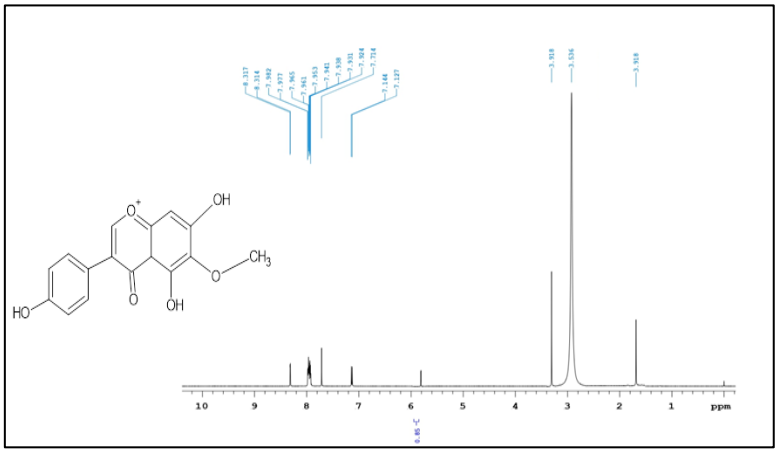

Figure No. 2: ^{1}H-NMR spectrum of compound 1

^{1}H NMR: δ 1.50-1.77 (4H, 1.58 (ddddd, *J* = 13.0, 4.2, 3.9, 1.9, 1.7 Hz), 1.62 (ddddd, *J* = 12.9, 3.9, 3.5, 2.1, 1.9 Hz), 1.66 (ddddd, *J* = 12.9, 10.1, 10.0, 3.6, 1.9 Hz), 1.68 (dtdd, *J* = 13.0, 9.9, 4.2, 1.9 Hz)), 2.55-2.85 (4H, 2.64 (ddd, *J* = 13.6, 10.1, 3.5 Hz), 2.68 (ddd, *J* = 13.6, 3.6, 2.1 Hz), 2.75 (ddd, *J* = 13.5, 4.2, 1.7 Hz), 2.76 (ddd, *J* = 13.5, 9.9, 4.2 Hz)), 5.79 (1H, s), 7.18 (1H, tt, *J* = 7.8, 1.3 Hz), 7.25-7.45 (6H, 7.32 (dddd, *J* = 8.2, 7.8, 1.4, 0.5 Hz), 7.36 (td, *J* = 8.5, 1.5 Hz), 7.38 (td, *J* = 8.5, 1.5 Hz), 7.39 (dddd, *J* = 8.2, 1.5, 1.3, 0.5 Hz).

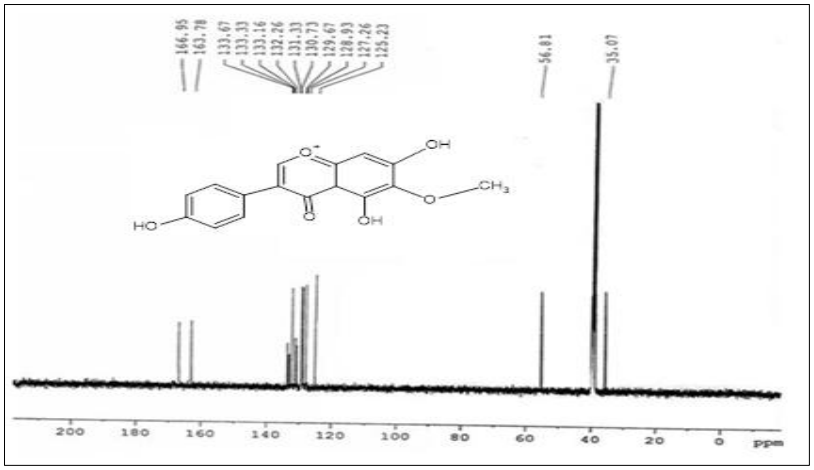

Figure No. 3: ^{13}C NMR spectrum of compound 1

^{13}C NMR: δ 40.7 (1C, s), 57.1 (1C, s), 71.7-71.8 (2C, 71.7 (s), 71.7 (s)), 115.7 (2C, s), 123.8 (1C, s), 127.3 (1C, s), 130.0 (2C, s), 131.7 (1C, s), 146.4 (1C, s), 152.4-152.6 (2C, 152.5 (s), 152.5 (s)), 157.4 (1C, s), 194.7 (1C, s).

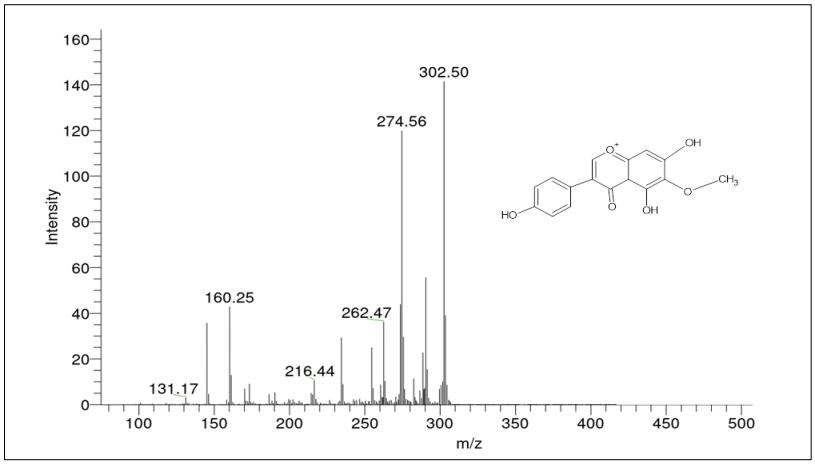

Figure No. 4: MS spectrum of compound 1

Observed Molecular Weight Found: 302.50 (M+H).

Chinese Journal of Health Management
Volume 20 Issue 5, Year of Publication 2026, Page 1164-1169 | DoI-10.564220/1674-0815.2026.140

Compound II

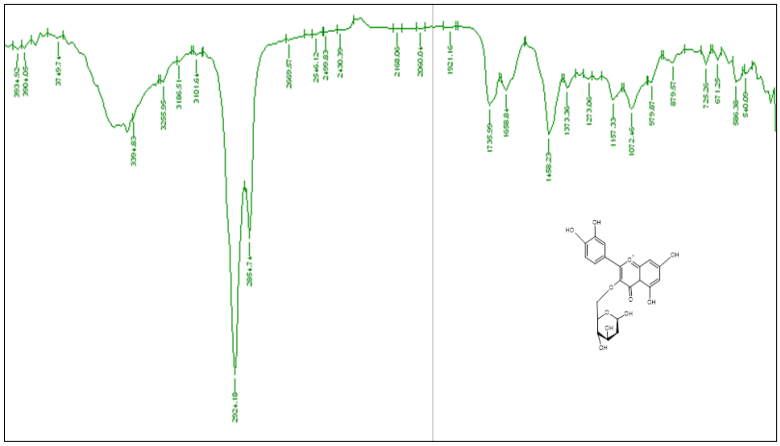

Figure No. 5: IR spectrum of compound II

IR (cm^{-1}) KBr: 3394.83 (O-H stretching), 2924.18 (C-H stretching), 3285.95(HC=CH stretching), 1736.99 (C=O Stretching), 1658.84(C=C), 1458.23(CH from CH2), 1373 [C-H stretching (CH2 (CH3)2)], 1157.33(OH-bending) 1072.46 (C-C stretch)

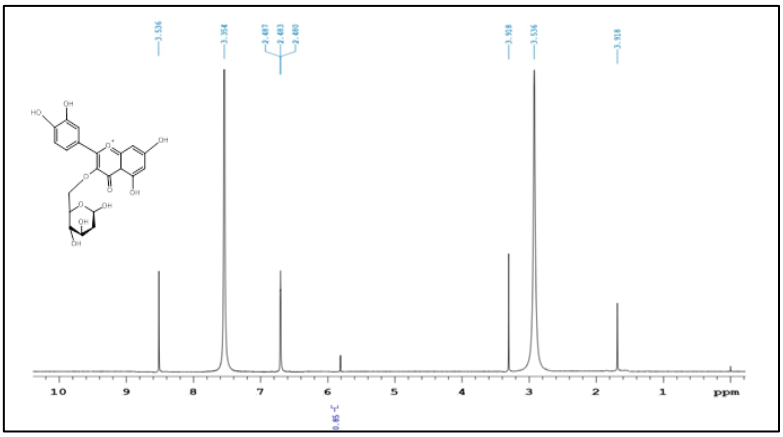

Figure No. 6: ^{1}H-NMR spectrum of compound II

^{1}H NMR: δ 1.50-1.77 (4H, 1.58 (ddddd, *J* = 13.0, 4.2, 3.9, 1.9, 1.7 Hz), 1.62 (ddddd, *J* = 12.9, 3.9, 3.5, 2.1, 1.9 Hz), 1.66 (ddddd, *J* = 12.9, 10.1, 10.0, 3.6, 1.9 Hz), 1.68 (dtdd, *J* = 13.0, 9.9, 4.2, 1.9 Hz)), 2.55-2.85 (4H, 2.64 (ddd, *J* = 13.6, 10.1, 3.5 Hz), 2.68 (ddd, *J* = 13.6, 3.6, 2.1 Hz), 2.75 (ddd, *J* = 13.5, 4.2, 1.7 Hz), 2.76 (ddd, *J* = 13.5, 9.9, 4.2 Hz)), 5.85 (1H, s), 7.19 (1H, tt, *J* = 7.8, 1.3 Hz), 7.25-7.46 (4H, 7.32 (dddd, *J* = 8.1, 7.8, 1.4, 0.5 Hz)

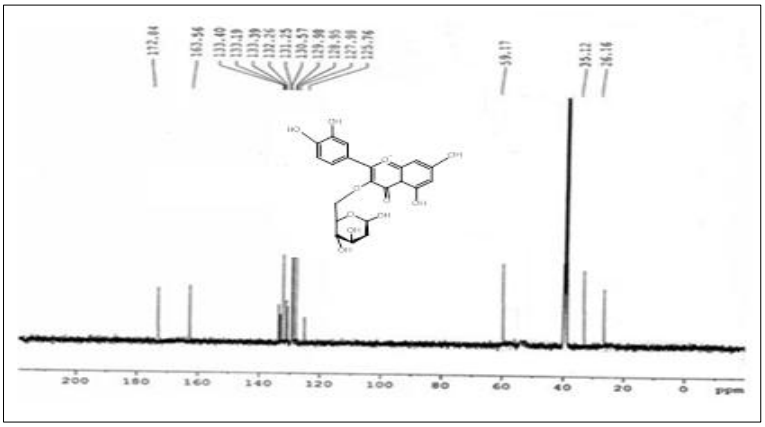

Figure No. 7: ^{13}C NMR spectrum of compound II

^{13}C NMR: δ 37.5 (1C, s), 40.7 (1C, s), 65.7 (1C, s), 71.7-71.8 (2C, 71.7 (s), 71.7 (s)), 72.3 (1C, s), 75.7 (1C, s), 76.7 (1C, s), 93.1 (1C, s), 96.8 (1C, s), 110.6 (1C, s), 115.8 (1C, s), 123.8 (1C, s), 127.8 (1C, s), 128.0 (1C, s), 145.8 (1C, s), 146.3 (1C, s), 152.4-152.6 (2C, 152.5 (s), 152.5 (s)), 161.8 (1C, s), 180.4 (1C, s).

Chinese Journal of Health Management
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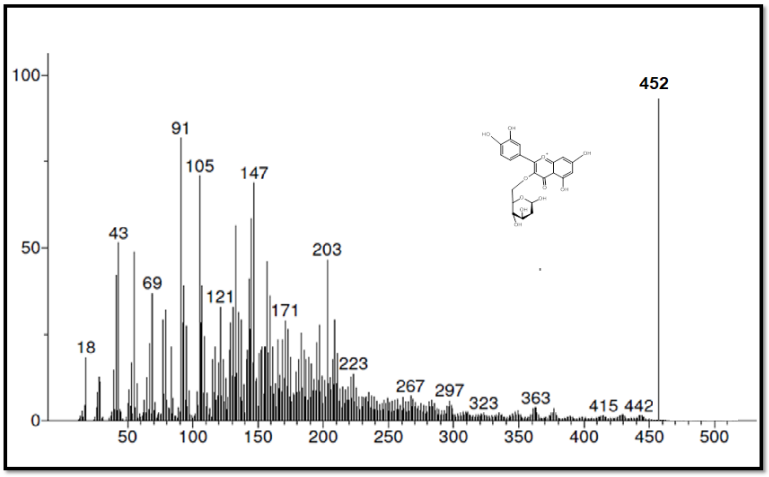
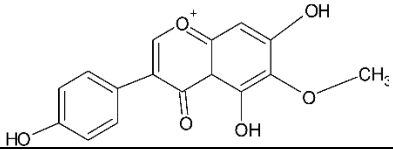
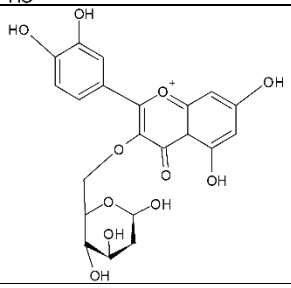


Figure No. 8: MS spectrum of compound II

Observed Molecular Weight Found: 452 (M+H).

RESULT DETAILS OF OBSERVED COMPOUND STRUCTURES:

Table No. 2: Test compound structure, IUPAC name, Molecular Formula and Molecular weight

Compound Number	Chemical Structure	IUPAC
Compound I **FLE-B3**		5,7-dihydroxy-3-(4-hydroxyphenyl)-6-methoxy-4-oxo-4,4a-dihydro-1-benzopyran-1-ium **Molecular Formula:** $C_{16}H_{13}O_6$ **Molecular weight:** 302.50
Compound II **ALE-D4**		2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[[(4S,6R)-3,4,6-trihydroxyoxan-2-yl]methoxy]-4aH-chromen-1-ium-4-one **Molecular Formula:** $C_{21}H_{21}O_{11}$ **Molecular weight:** 449.38 (452)

CONCLUSION:

The structural elucidation of the lead bioactive fractions confirms the presence of two potent flavonoid derivatives, which validates the significance of antimicrobial and antioxidant activities. The identification of these specific chromen-1-ium (flavylium) derivatives provides a definitive chemical basis for the therapeutic potential of ***Feronia limonia*** and ***Aegle marmelos*****.** The synergy between the methoxy groups and the polyhydroxylated frames explains the enhanced efficacy of these fractions over standard antioxidants. These molecules represent promising lead candidates for further pharmacological development as natural neuroprotective therapies as a lead anticonvulsant agent.

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