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ISOLATION OF TRITERPENES FROM *FIORIA VITIFOLIA* (L.) AND IT'S ANTIOXIDANT ACTIVITY

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ABSTRACT: *Fioria vitifolia* L. (Linn.), (Malvacae) has been extensively used in folk medicine for the treatment of common cold, flu, and upper respiratory infections and also used as an immune system booster. The methanolic extract of whole plant of *Fioria vitifolia* was taken under phytochemical investigation. The methanolic extract was partitioned with different solvent system by increasing their polarities using column chromatography, followed by thin layer chromatography for spot identification. Steroids, flavonoids, and triterpenes, gossypin alkaloids have been isolated from the whole part of the plant .The methanolic extract of dried plant of *Fioria vitifolia* (MEFV) was investigated for anti-inflammatory (carrageenan induced rat paw oedema)and anti-pyretic(brewer's yeast induced pyrexia)and anti-oxidant activities. *It* is used in traditional medicine for the relief of pain and inflammation in Tamil nadu, India. Preliminary studies showed the analgesic activity of methanol extract of aerial parts of the plant against Eddy's hot plate and tail flick models and anti-inflammatory activity against carrageenan-induced model in albino rats. In this follow up studies, the major active principle was isolated by column chromatography and identified as friedelin and epifriedelinol using spectral data. Friedelin showed promising antioxidant activity against Nitric oxide radicals *in vitro*. Further, this study revealed the presence of friedelin and epifriedelinol in *Fioria Vitifolia* L plant.

INTRODUCTION: *Fioria vitifolia* (L.) Mattei belonging to family Malvaceae commonly known as *Hibiscus Vitifolius* is a plant native to India [1].

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Traditionally, the plant is used as analgesic, anti-inflammatory, anti-pyretic, UTI, astringent, sedative, CNS depressant, anti-fertility, anti-bacteria.

Various extract of the whole plant are used in herbal medicines to treat pain, fever and inflammation. The whole plant extract was proved to be as effective as aspirin in rats.

MATERIALS AND METHODS:

Plant Materials:

General experimental procedures: The melting points were determined with Toshniwal apparatus and were uncorrected. IR spectra were recorded on a Perkins-Elmer GX FTIR instrument using potassium bromide pellets and sodium chloride cells. NMR spectral analyses were carried out with JEOL FT-NMR 400 ECP Spectrometer at 400 MHz (1H) and 100 MHz (13C). The samples were dissolved in $CDCL_3$ and chemical shifts (in ppm) were referenced to TMS for 1H NMR. Mass spectra were measured using HP LC-MS.

Thin Layer Chromatography (TLC) was carried out on pre-coated silica gel 60 F254 TLC plate (Merck). The plates were visualized under ultraviolet light at λ_{max}- 254 nm and also spraying the plates with 10% sulphuric acid. The conventional column chromatography was done using Merck silica gel 60 (230-400 mesh) while the vacuum liquid chromatography was done over silica gel 60 GF254.

Plant material:

Collection and identification: Ethno-medical survey was conducted in the bushy areas of Kallankattu valasu near Komarapalayam, Namakkal, Tamilnadu State, India to collect information on folk or traditional use of the plant *Fioria vitifolia* (L.). The survey brought to light the traditional use of *Fioria vitifolia* (L.) as analgesic, anti-inflammatory, anti-pyretic, UTI, astringent, sedative, CNS depressant, anti-fertility and anti-bacterial. The plant belongs to shrub and the leaves are rounded at the base. The flowers are single in leaf axis and it is pale yellow in colour and dark purple in the centre.

The identification of the collected plant was done in southern regional centre of Tamilnadu Agricultural University campus at botanical survey of India, Coimbatore, Tamil Nadu.

Extraction and isolation:

Extraction: Plant material was powdered in to coarse and extracted successively with Petroleum ether, Chloroform, Ethyl acetate and Methanol by using soxhlet extractor. The extracts were concentrated by using rotary evaporator and dried. The yield of all the extracts was noted. These extracts were used for isolation of pure compounds.

Chromatographic separation: Chromatographic separation of Petroleum ether extract (10gm) was performed using silica gel (60:100) packed column chromatography. Elution was carried out by using different solvent system of increasing polarity i.e. Petroleum ether, Chloroform, Ethyl acetate and Methanol in different ratios. All the fractions (50ml) were collected in boiling test tubes and checked by thin layer chromatography. Fractions showed identical spots were pooled for further purification. The fraction numbers 32 and 33 collected with petroleum ether and chloroform in the ratios of 80:20 was showed single spot in TLC (Pet. ether: Chloroform 50: 50) and pooled. Further the residue was obtained after evaporation was purified by recrystallization using hexane solvent. The compound isolated was checked for its polarity using TLC by various solvent systems and named as Compound I.

The fractions 34, 35 and 36 collected in Pet. ether: Chloroform 60: 40 ratio were showed similar single spots in TLC, but different Rf value with previous 32 & 33 fractions were mixed. Again the residue was recrystallized, checked its purity and named as Compound II. These compounds are characterized by UV, IR, Mass and NMR spectroscopy for their structural confirmation.

Nitric oxide Scavenging Activity: Nitric oxide was estimated spectrophotometrically by the method at 546 nm [2]. Sodium nitroprusside (5mM) in phosphate buffered saline was mixed with different concentrations of friedelin (10-100 µg/ml) dissolved in methanol and incubated at 25°C for 30 min. Then 1.5 ml of the incubated solution was removed and diluted with 1.5 ml of Griess reagent (1% Sulfanilamide, 2% phosphoric acid, and 0.1% naphthyl ethylene diamine dihydrochloride).

The absorbance of the chromophore formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with naphthylethylene diamine was measured at 546 nm. Percentage scavenging activity (antioxidant activity) can be calculated by the following formula;

% Antioxidant activity =

$$\frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

RESULTS AND DISCUSSION:

Characterization of compound I:

IR cm^{-1} **(KBr):** 3417 (ν c-O-H, Hydroxyl group), 2919, 2849 (ν C-H, aliphatic), 1735 (ν C=O of terpenoids), 1619 (ν C=C of Lupeol or glutinol or beta amyrin as an impurity), 1466 (ν CH_2), 1400(ν C-OH) and 1110 (ν C-O-C).

^{1}H NMR ($CDCl_3$): The three protons of methyl group at C_{23} attached to C_4 carbon and located adjacent to a hydroxyl group at C_3 appeared as a singlet at δ 2.63 (3H, s, C_4). The seven other methyl groups, appeared as a most intense singlet signal at δ 1.27 (21H, s, C_{24}, C_{25}, C_{26}, C_{27}, C_{28}, C_{29} & C_{30}). The three methylene protons (6H, d, C_1, C_7 & C_{19}) appeared as doublet between δ 3.66-3.67 by coupling with his C-H methine neighbor (C_{10}, C_8 & C_{18}). The possible coupling pattern may be C_7 proton coupled with C_8 proton, C_1 proton coupled with C_{10} proton, C_{19} proton coupled with C_{18} proton.

The other seven methylene group protons (16H, m, C_6, C_{11}, C_{12}, C_{15}, C_{16} C_{21} & C_{22}) appeared as a strong multiplet signal between 3.54-3.58. The methylene protons attached to C_2 appeared as doublet at 3.66-3.67. The presence of three methine (3H, t, $C_{8,}$ C_{10} &C_{18}) groups, are clearly evident from the triplet signal between δ 4.05-4.08 (triplet, 3H, $C_{8,}$ C_{10} &C_{18}). The presence of single methine (CH, t, C_3) group sharing the hydroxyl group attached carbon at C_3 clearly evident from the triplet signal between δ 4.05-4.08

^{13}C NMR ($CDCl_3$): In ^{13}C NMR spectra, the ketonic carbon is absent between δ 170-210 at C_3 appeared as a doublet at δ 29.48 indicating the presence of a single proton attached to C_3 carbon. The eight methyl groups, appeared at δ 25.95-C_{23}, 17.46-C_{24}, 22.69-C_{25}, C_{26}, C_{27}, C_{28}, 14.10-C_{29} & C_{30}). The methylene carbon at C_2 appeared at δ 31.93, the other ten methylene carbons (C_1, C_6, C_7, C_{11}, C_{12}, C_{15}, C_{16} C_{19}, C_{21} & C_{22}) appeared as multiplets between δ 29.48-29.70. The three methine carbons (C_8, C_{10} & C_{18}) appeared as a doublet between δ 29.26-29.36.

Mass spectrum of LC retention time 0.619: The molecular ion peak appeared at M^+ 428 and the base peak appeared at 157. The most characteristic fragments are 96, 179, 210, 242, 277, 309 and 342, 372 and 414. From the spectral data such as IR, ^{1}HNMR, **^{13}C NMR** and mass spectra, the molecular formula for compound I was suggested as $C_{30}H_{52}O$ from EI mass spectrum which gave the molecular ion peak at m/z 428. The IR spectrum showed an intense band at 1735 cm^{-1} consistent with a six membered ring ketone. Since the molecular formula indicated six units of saturation, this compound was concluded to be a pentacyclic triterpene with a hydroxyl group at 3417 cm^{-1} in infra-red spectrum. The presence of signals in proton and carbon NMR spectrum suggested the **Friedelane skeleton**. The melting point was recorded as m.p. 280-282°C [3].

Based on the spectral data, compound I was elucidated as epi-friedelinol and the structure was confirmed as **Figure 1** by comparison of its physical properties and proton, carbon NMR data to literature [4 & 5].

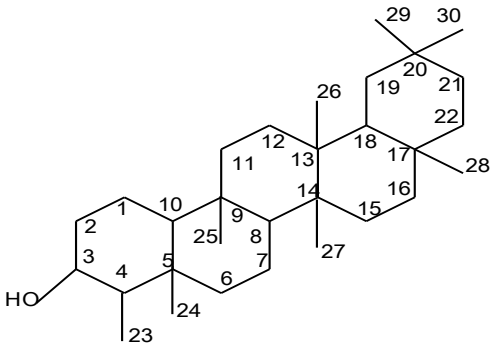

FIGURE 1: STRUCTURE OF EPI-FRIEDELINOL
MF: $C_{30}H_{52}O$; MW: 428; M.P. 280-282°C

Characterization of compound II:

IR cm^{-1} **(KBr):** 3416 (ν c-O-H of epifriedelinol as an impurity), 2910, 2848 (ν C-H, aliphatic), 1735 (ν C=O of terpenoids), 1618 (ν C=C of Lupeol or glutinol or beta amyrin as an impurity), 1467 (ν CH_2), 1172-1112 (ν C-O-C) and 1016(ν c-O-H).

^{1}H NMR ($CDCL_3$): The three protons of methyl group at C_{23} attached to C_4 carbon and located adjacent to a ketone group at C_3 appeared as a singlet at δ 2.63 (3H, s, C_4). The seven other methyl groups, appeared as a most intense singlet signal at δ 1.27 (21H, s, C_{24}, C_{25}, C_{26}, C_{27}, C_{28}, C_{29} & C_{30}).

The three methylene protons (6H, d, C_1, C_7 & C_{19}) appeared as doublet between δ 3.66-3.67 by coupling with his C-H methine neighbor (C_{10}, C_8 & C_{18}). The possible coupling pattern may be C_7 proton coupled with C_8 proton, C_1 proton coupled with C_{10} proton, C_{19} proton coupled with C_{18} proton. The other eight methylene group protons (16H, m, C_2, C_6, C_{11}, C_{12}, C_{15}, C_{16} C_{21} & C_{22}) appeared as a strong multiplet signal between 3.53-3.58. The presence of three methine (3H, t, C_8, C_{10} &C_{18}) groups, are clearly evident from the triplet signal between δ 4.06-4.08 (triplet, 3H, C_8, C_{10} & C_{18}),

^{13}C NMR ($CDCL_3$): In ^{13}C NMR spectra, the ketonic carbon at C_3 appeared as a singlet at δ174. The eight methyl groups, appeared at δ 25.95-C_{23}, 17.46-C_{24}, 22.69-C_{25}, C_{26}, C_{27}, C_{28}, 14.10-C_{29} & C_{30}). The methylene carbon at C_2 appeared at δ 31.93, the other ten methylene carbons (C_1, C_6, C_7, C_{11}, C_{12}, C_{15}, C_{16} C_{19}, C_{21} & C_{22}) appeared as multiplets between δ 29.48-29.70. The three methine carbons (C_8, C_{10} & C_{18}) appeared as a doublet between δ 29.26-29.28.

LC-EI-MS: The retention time of the compound was recorded as 0.698. The molecular ion peak appeared at M^+ 426 and the base peak appeared at 157. The most characteristic fragments are 85, 123, 205, 218, 273, 306 and 341.

From the spectral data such as IR, ^{1}HNMR, **^{13}C NMR** and mass spectra, the molecular formula for compound II was suggested as $C_{30}H_{50}O$ from EI mass spectrum which gave the molecular ion peak at m/z 426. The IR spectrum showed an intense band at 1735 cm^{-1} consistent with a six membered ring ketone.

Since the molecular formula indicated six units of saturation, this compound was concluded to be a pentacyclic triterpene with a ketone group. The presence of signals in proton and carbon NMR spectrum suggested the friedelane skeleton. The melting point was recorded as m.p. 259-261°C [6].

Based on the spectral data, compound II was elucidated as friedelin and the structure was confirmed as **Figure 2** by comparison of its physical properties and proton and carbon NMR data to literature [7& 8].

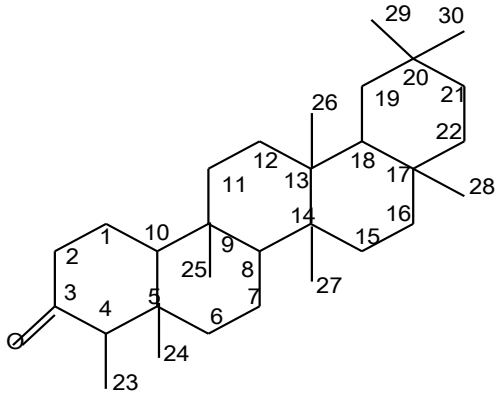

FIGURE 2: STRUCTURE OF FRIEDELIN

MF: $C_{30}H_{50}O$; MW: 426; M.P. 259-261°C

Nitric oxide Scavenging Activity: In nitric oxide scavenging activity study, Friedelin proved to be a good nitric oxide radical fighter by its showing IC_{50} value 76.96µg/ml. The results were compared with standard antioxidant ascorbic acid whose IC_{50} value was calculated as 20.22µg/ml. The maximum activity was recorded as 62.23% inhibition for the concentration of 100 µg/ml compared to the standard, 91.87% for the same concentration [9-15]. The results are presented in **Table 1**.

TABLE 1: EFFECT OF FRIEDELIN ON NITRIC OXIDE RADICAL

Compound	Conc. (µg/ml)	Absorbance At 546 nm	% NOR inhibition	IC_{50} values
Nitric oxide Control	-	0.985	-	
Friedelin	10	0.855	13.19	76.96 µg/ml
	20	0.768	22.03	
	40	0.702	28.73	
	60	0.552	43.95	
	80	0.473	51.97	
	100	0.372	62.23	
Vitamin-C	10	0.791	19.69	20.22 µg/ml
	20	0.498	49.44	
	40	0.385	60.91	
	60	0.263	73.29	
	80	0.195	80.20	
	100	0.080	91.87	

CONCLUSION: The chemical component of *Fioria vitifolia* L. is investigated for the first time which resulted in the isolation of Friedelin and epi-friedelinol. The nitric oxide scavenging activity proves its role in mediating the mechanism of analgesic activity [16-19].

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