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Structural Characterization and Pharmacological Property of Compound Fernolin from *Feronia Limonia*: A Review

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Abstract: ***Plant serve as vast source for many phytoconstituents which exhibit pharmacological property. Identification of these types of potential plants is of significance in medicine. Therefore it is necessary to study the pharmacognostic characteristic of the plant before using in the field of research and pharmaceutical formulation. Feronia limonia plant is well known for its medicinal properties. Wood apple has antioxidant, anticancer, antidiabetic, antimicrobial and hepato-protective activity. A monoterpenoid-5-methoxyfuranocoumarin lactone, named as Fernolin or Ferolactone was isolated from root and fruit of Feronia limonia. Fernolin show photosensitizing effects and antihepatotoxic activity.***

Keywords: *F. limonia*, Furanocoumarin, Spectral analysis, Pharmacological property.

1. Introduction

Human beings have been dependent on higher plants for their health care needs since the very beginning of human civilization. In addition to food, clothing and shelter, the green plants have provided all the medicaments to man and domestic animals for thousands of years. From folk medicine and traditional system of medicine, medicinal plants were adopted into modern system of medicine after they have been found effective drugs through chemical and pharmacological screening. The medicinal value of drug plants is due to presence of some chemical substances in the plant tissue which produce a definite physiologicalaction in the human body. The most important chemical substance are alkaloids, terpenes, sterols, glycosides, saponins, gums, fatty acids, lactones, coumarins, carbohydrates, essential oils, waxes, amino acids, proteins, tannins, enzymes, resins, hydrocarbons, coloring matters, aliphatic ketones, esters and alcohols etc.

Feronia limonia is one of the medicinal plant which is commonly known as Kaitha in Hindi and Wood apple in English. It is widely distributed in deciduous and arid landscapes of several countries. In India it is found throughout the planes, particularly in dry situations of Aravallis in south-east Rajasthan and also up to an elevation of 1500 feet in western Himalayas. *Feronia limonia* is moderate-sized tree belongs to the family Rutaceae. Many parts of this plant such as leaves, barks, fruits, roots and gums have been prescribed in traditional medicine for man ailments. In vitro and in vivo studies have supported the ancient use of *Feronia limonia* in various diseases. A variety of bioactive compounds like phenols, flavonoids, alkaloids, terpenoids, tannins, saponins, coumarins, fat steroids, glycosides, gum mucilage and essential oil have been reported in different extracts of wood apple which are responsible in various pharmacological activities. A variety of pharmacological activities such as antitumor, antimicrobial, antidiabetic, anti-inflammatory, analgesic, antioxidant, hepatoprotective, antimutagenic, antimalarial and other activities has been reported from extract of different parts of this plant. Fernolin is a furanocoumarin which has been isolated from root and fruitof this plant. Agarwal A *et al*.[1]The main objective to study the chemical structure of isolated plant constituents Fernolin is that it may be responsible for their pharmacological activity.

The procedure generally used for the isolation of the compounds was thin layer chromatography and column chromatography. Structure of the compounds isolated, was established mainly on the basis of spectral evidences i.e. UV, IR, ^{1}H NMR, Mass and ^{13}C NMR.

2. Methodology

The air-dried and finely crushed plant material (5kg) of *Feronia limonia* was extracted with ethanol. The ethanolic extract on keeping over night, deposited a dirty residue, which was separated by filteration. The filtrate was concentrated and poured into large excess of ice cold water with constant stirring. A reddish brown aqueous solution and a dark brown water insoluble residue were separated.

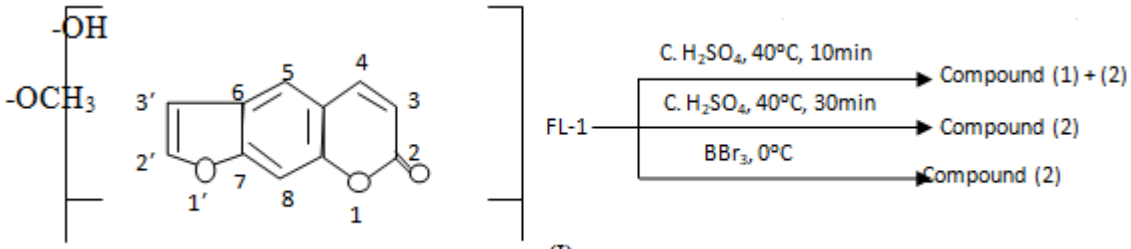
The water soluble fraction was concentrated in rotary evaporator and fractioned by liquid-liquid extractor with different solvents in increasing order of polarity, viz, hexane, benzene, ether and ethyl acetate, respectively. From ethyl acetate extract, on keeping for about two days at low temperature, after concentration, a white compound FL-1, m.p. 262ºC, was isolated after repeated crystallization from dichloromethane.

3. Result and Discussion

The compound FL-1, m.p.262ºC was analyzed for $C_{22}H_{20}O_7$ on the basis of molecular weight determination, (M^+ 396). The compound gave blue fluorescence under UV light. Chatterjee et al.[7]The linear furanocoumarin nature of compound was confirmed by its UV absorption maxima at 220, 252, 276 and 312nm, as furanocoumarin absorb in the same region. Chakraborty D P.[8] IR absorptions at 1755 and

1710cm^{-1} indicated the presence of α, β-unsaturated γ-lactone and α, β-unsaturated δ-lactone. Laxmi et al.[9] The presence of methoxyl and methyl groups was inferred by bands at 2920 and 2800 cm^{-1} in its IR spectrum.

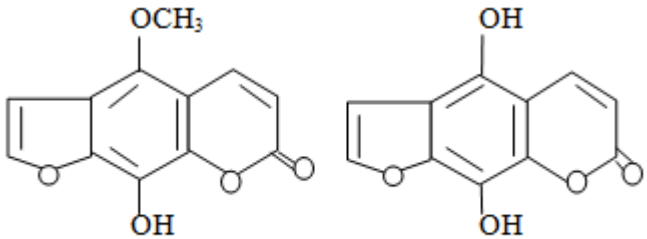
On treatment with conc. H_2SO_4 for 10minutes at 40ºC, it afforded compound(1) as a major product whereas for 30minutes compound (2) was the only product and on treatment with BBr_3 at 0ºC the compound FL-1 afforded only compound (2).



(I)

Four signals corresponding to one proton were present in the aromatic region of the compound (1) suggesting the disubstituted furanocoumarin. A pair of doublets at δ6.30 and 7.76ppm (*J*=9Hz) in its ^{1}H NMR spectrum was attributed to the pyran ring protons at C-3 and C-4, respectively. Another pair of doublets appeared at δ7.68 and 6.81ppm, (*J*=2.3Hz) corresponding to 2′- and 3′-furan rings protons. A sharp singlet atδ4.25ppm corresponded to methoxyl group which was presented at C-5 position. So, the position left for hydroxyl group was C-8 position.

Thus, the structure assigned to the compound (1), 5-methoxy-8-hydroxyfuranocoumarin, has been represented as II. The structure of copound (1) was also confirmed by its m.p. 214ºC and co-chromatography with an authentic sample.Abu-Mustafa et al[11]. Compound (2) was found to be 5,8-dihydroxyfuranocoumarin on direct comparison with an authentic sample (m.p. 210ºC). Abu-Mustafa et al.[11]Structure of compound (2) could, thus, be represented as III.



(II) (III)

On the basis of above evidences, it is clear that the compound (1) was present as the nucleus in the compound FL-1 or in other word, the compound FL-1 was ether derivative of compound (1). The 200MHz spectrum of the compound FL-1 integrated for 20 protons and assignments of their chemical shifts are given in the table I.

A pair of doublets of δ6.35 (1H,*J*=9.5Hz) and 7.76 (1H, *J*=9.5Hz)ppm was assigned to the C-3 and C-4 protons of the coumarin nucleus. Such doublets were characteristic of the unsubstituted pyran nucleus. Another characteristic pair of doublets for the protons C-2′ and C-3′ appeared at 7.71 (1H, *J*=2.3Hz) and 6.80 (1H, *J*=2.3Hz)ppm. A doublet of two protons which appeared at δ5.09 (*J*=6.6Hz) was assigned to the –OCH_2– group at C-1″. The olefinic proton at C-2″ appeared as multiplet at δ5.72ppm (*J*=6.6 and 0.98Hz) due to the coupling effect of methylene and methyl protons at C-1″ and C-4″, respectively. The three methyl protons at C-4″ appeared as broad singlet at δ1.79 and methylene group at C-5″appeared as multipet at δ2.36ppm. The multiple centered at δ4.92ppm (*J*=6.5 and 1.95Hz) was assigned to the protons at C-6″ of a five-membered lactone. Signal for H-7″ appeared as multiplet at δ6.93ppm (*J*=1.71 and 0.16 Hz) coupled by H-6″ (methyne proton) and H-9″ (methyl proton). The triplet at δ1.88ppm (*J*=1.71 and 1.95 Hz) for three protons could be assigned to the methyl function at the α-position of the α, β- unsaturated γ-lactone group. The multiplicity being explained by the assumption of the long range homo-allylic with H-6″.

Compound (1): The UV absorption maxima of the compound (1) at 220,242 (sh), 250 and 314nm was in close agreement with that reported for 5,8-dioxygenated furanocoumarin. Murray R D H et al.[10]The ^{1}H NMR spectrum revealed the presence of one methoxyl [δ4.25ppm (s, 3H)] and one hydroxyl [δ6.20ppm (s, -OH)] function. Compound (1) can be represented as I.

Melting point	262ºC
Elemental analysis	Found C : 65.26% H : 5.01% / Calculated for $C_{20}H_{20}O_7$ C : 66.66% H : 5.05%
UV λ_{max}^{MeOH}	220, 252, 276, 312 nm
IR ν_{max}^{KBr}	2920, 2800, 1755, 1710, 1615, 1590, 1440, 1400, 1325, 1290, 1210, 1180, 1150, 1025 cm^{-1}
^{13}C NMR ($CDCl_3$)	δ160.37 (C-2), 114.76 (C-3), 144.32(C- 4 & C-7″), 116.58 (C-4a), 131.0 (C-5), 125.95(C-6), 148.26 (C-7), 131.48(C-8), 148.65 (C-8a), 146.70 (C-2′), 106.85 (C-3′), 69.69 (C-1″), 123.94 (C-2″), 137.02(C-3″), 10.58 (C-4″), 43.37 (C-5″), 79.54 (C-6″), 130.8 (C-8″), 17.24 (C-9″), 173.82 (C-10″), 63.00 (-OCH_3)ppm.
Mass spectra, m/z	396(M^+), 309, 232, 204, 165, 97, 69, 41

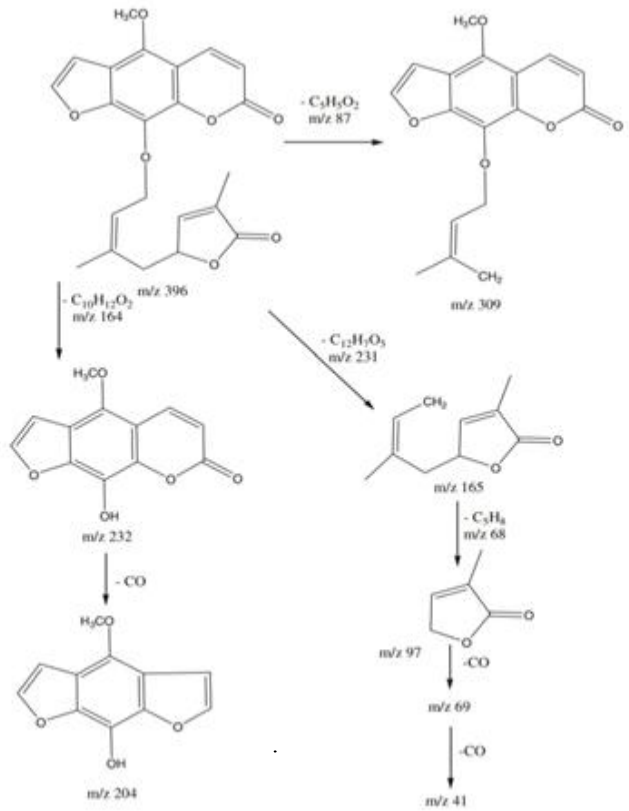
Assignments	Chemical Shifts (δ-ppm)	*J* values (Hz)
H-2′	7.71(d, 1H)	2.3
H-3′	6.80(d, 1H)	2.3
H-3	6.35(d, 1H)	9.5
H-4	7.76(d, 1H)	9.5
H-1″	5.09 (d, 2H)	6.6
H-2″	5.72(tm, 1H)	6.6 & 0.98
H-4″	1.79(brs, 3H)	-
H-5″	2.36(m,2H)	6.5
H-6″	4.92(tm, 1H)	6.5 & 1.95
H-7″	6.93(dq, 1H)	1.71& 0.16

H-9″	1.88(t,3H)	1.71 & 1.95
-OCH_3	3.90(s,3H)	

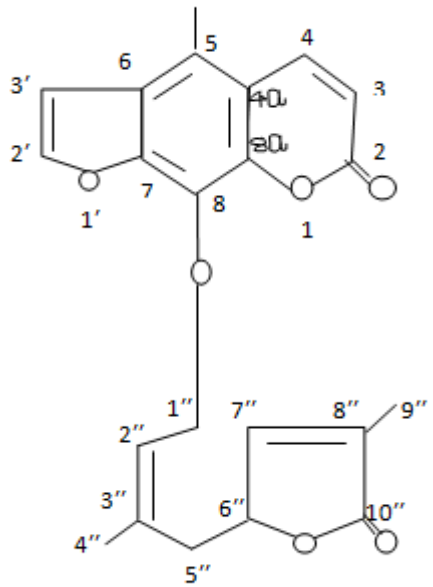
The structure of this compound was confirmed by decoupling experiment (NMDR technique). The relationship of H-9″ with H-6″ and H-7″ was established by decoupling experiment. Irradiation at δ 1.8866ppm caused the multiplet (dq at δ6.93, H-7″) change to a doublet accompanied by a simultaneous change in the shape of the multiplet (tm at δ4.92, H-6″). Irradiation at δ2.3648 simplified the multiplets at δ4.92 (H-6″) and 6.93 (H-7″) establishing the relationship of H-5″, H-6″ and H-7″. This was further supported by the appearance of sharp singlet at δ1.88ppm (H-9″), add at δ2.36 (H-5″) and simplification of the multiplet at δ6.93 (H-7″) by irradiation at δ4.9254. Irradiation at δ5.0416 simplified the multiplet at δ5.72 (H-2″) showing the relationship between H-1″ and H-2″ as supported by the change of doublet at δ5.09 (H-1″) on irradiation at δ5.7267. Irradiation at δ6.9342 changed the double doublet appearing like the triplet at δ1.88(H-9″) to doublet accompanied by simultaneous change in the shape of multipet at δ4.92 (H-6″). On the basis of the above findings, the structure of the compound FL-1 as fernolin has been represented as (IV)

The structure of the compound FL-1 as IV was also confirmed by ^{13}C NMR data.Laxmi et al.[9]

The ^{13}C NMR of the compound showed 21 signals for 22 carbons. The signals at δ10.58 and 17.24 ppm were assigned to the methyl carbon at C-4″ and C-9″, respectively. The signal at δ43.37 could be assigned to the methylene carbon (C-5″) while another –CH_2- carbon (C-1″) appeared appreciably downfield at δ69.69ppm showing its linkage with oxygen. Similarly, another saturated carbon C-6″ appeared at δ79.54ppm because of the adjacent oxygen atom (ether linkage). The signals at δ106.85 and 146.70 were assigned to C-3′ and C-2′, respectively. The two unsubstituted carbon in the coumarin ring appearing at δ114.76 (C-3) and 144.32 (C-4) shifted to a doublet by OFR (off field resonance) experiment. Ring carbons appearing at δ116.58 (C-4a), 148.65 (C-8a), 125.95 (C-6), 148.26 (C-7) and 131.48 (C-8) did not show coupling, which confirmed the absence of proton on them. Aromatic carbon at position C-5 appearing at δ131ppm also showed no doublet with OFR, confirming that position-5 was substituted. Peaks for C-5, C-7, C-8 and C-8a appeared downfield as compared to C-4a and C-6 because of the oxygenation at C-5, C-7, C-8 and C-8a. Olefinic carbons appearing at δ123.94 and 137.02 were assigned to C-2″ and C-3″. Signal for C-7″ appearing together with C-4 at δ144.32 also showed a doublet by OFR experiment. The two-signals for low intensity at δ160.37 and 173.82 were assigned to the carbonyl carbons, C-2 and C-10″, respectively. A signal for C-8″ appeared at δ130.8ppm and a signal appeared at δ63.00 for methoxyl group at C-5.



Mass Scheme I



(IV)

The size and the nature of the side chain were assessed by the study of its mass spectrum. Mass spectral data showed base peak at m/z 232 and molecular ion peak at m/z 396. Appearance of the ions at m/z 309 and 165 were diagnostic of the side chain attached to oxygen at C-8 of the furanocoumarin nucleus. The presence of a base peak at m/z232 and ion at m/z 204 provided sufficient evidence for the presence of furanocoumarin nucleus. The ion at m/z 97 was attributable to γ-lactone moiety of the side chain. The fragmentation pattern has been represented in mass scheme I.

Dealkylation of compound FL-1

Method 1: To the compound (0.1g) dissolved in acetic acid (1ml), two drops of conc. H_2SO_4 were added. The reaction mixture was heated for 10 min (40ºC), cooled, diluted with ice water and extracted with ethyl acetate. The ethyl acetate part was washed with water, dried over anhydrous Na_2SO_4 and the solvent removed to afford the crude products. Purification over preparative TLC on silica gel yielded 5-methoxy-8-hydroxyfuranocoumarin, compound (1), m.p. 214ºC and 5, 8-dihdroxyfuranocoumarin, compound (2), m.p. 210ºC. When the reaction mixture was heated for 30min (40ºC) and worked up as usual, only compound (2) was obtained m.p. 210ºC.

Method 2: To a well-stirred solution of the compound (0.05g) in dichloromethane (20ml), borontribromide (0.05g) in dichloromethane (10ml) was added at 0ºC. The stirring was further continued at room temperature for 24hr. The solution was then poured into water and extracted with ethyl acetate. The crude product on crystallization gave a crystalline product, 5, 8-dihydroxyfuranocoumarin, m.p. 209ºC.

Compound (1)

m.p.	214ºC	
UV λ_{max}^{MeOH}	220, 242 (sh), 250, 314nm	
^{1}H NMR ($CDCl_3$, 90MHz):		
Assignments	Chemical shifts (δ-ppm)	*J* values (Hz)
-OH	6.20 (s, 1H)	
$-OCH_3$	4.25 (s, 3H)	
H-2′	7.68 (d, 1H)	2.3
H-3′	6.81 (d, 1H)	2.3
H-3	6.30 (d,1H)	9
H-4	7.76 (d, 1H)	9

Pharmacological Properties

The fruit, root, bark and leaves of *F. limonia* plant are used in the treatment of snake-bite (Charaka, Sushruta, Vagbhata). The fruit is recommended in scorpion sting (Charaka, Sushruta). The roots and fruit of *F.limonia* contain monoterpenoid-5-methoxyfuranocoumarin lactone, named as Fernolin or Ferolactone. Agarwal A et al.[1] Furanocoumarin show dermal photosensitizing effects i.e. the biological effects exerted by them upon irradiation with long wavelength UV light.Soine T O [2]. Application of this substance on the skin followed by exposure to sunlight causes erythema and pigmentation. Intense exposure may lead to hyper pigmentation and occasionally vesiculation of the skin. The substances responsible for this action on skin are furanocoumarin present in such plants. Dermatitis may be caused by some common plants or plant products such as Cologne water (containing bergamot oil), by rue, parsnip, fig leaves or celery. The best known photosensitizing effect of the furanocoumarins is erythema of human or guinea pig skin, appearing after the application of the substance on the skin followed by exposure to long wavelength UV light or sunlight. Erythema (edema and vasicle may also occur) appears after a latent period of several hr, lasts a few days and is followed by dark pigmentation. Kuske H, Musajo L et al., Fitzpatrick T B et al., Pathak M A et al.[3-6]

Ferolactone exhibited a significant antihepatotoxic activity against CCl_4-induced toxicity in Wistar rats in comparison with standard drug silymarin. Ferolactone exhibited a significant antihepatotoxic activity by reducing the elevated levels of serum enzymes such as serum glutamate oxaloacetate transaminase (SGOT) by 43.44%, serum glutamate pyruvate oxaloacetate transaminase (SGPT) by 39.63%, and alkaline phosphatase (ALP) by 41.16%, while the total protein (TP) levels were increased by 36.15%. When compared with standard drug, silymarin decreased SGOT by 45.80%, SGPT by 43.67%, ALP by 37.96% and increased TP levels by 55.16% against CCl4-induced toxicity in albino wister rats. These biochemical observations were also supplemented by histopathological examinations of the liver sections, which showed significant recovery of hepatocytes of the liver in ferolactone-treated animals. Upadhyay R et al.[12]

The pharmacological properties of *F. Limonia* fruit and root have been studied extensively in recent years. However, the active ingredients which exert respective pharmacological actions need to be identified and isolated so that the fruit and root may be used in medical treatment in the future.

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