CHAPTER 3
Phenylpropanoids from *Feronia elephantum* Correa
Estelar
3.1 INTRODUCTION

_Feronia elephantum_ Correa is an erect, slow growing, moderate sized tree, with rigid bark, fissured and scaly with sharp spines \(^1\). Leaves smelling of aniseed, alternate, imparipinnate; Petiole and rachis flat, leaflets 3-9, opposite 2-2.5 cm. Fruits 5-6.3 cm, globose and hard, paricarp woody, rough, gray coloured. Seeds embedded in an edible pulp \(^2\). The plant is the native and common in the wild plains of India and Ceylon up to 450 m. It is also cultivated in Bangladesh, Pakistan, Sri Lanka \(^3\) and often cultivated along roads edges or fields, occasionally in orchards \(^4\). The tree grows up to an elevation of 1,500 ft (450 m) in the Himalayan region \(^1\).

3.1.1 TAXONOMICAL CLASSIFICATION

- **Kingdom**: Plantae
- **Division**: Magnoliophyta
- **Class**: Magnoliopsida
- **Order**: Sapindales
- **Family**: Rutaceae
- **Genus**: Limonia, Feronia

3.1.2. SYNONYMS \(^5\)

_Limonia elephantum_ (Correa) Panigrahi, _Limonia acidissima_ L., _Schinus limonia_ L.

3.1.3 INDIAN NAMES \(^6\)

- **Eng.**: Wood Apple
- **Guj.**: Kotha, Kondhu
- **Hindi**: Kaitha
- **Punj.**: Kainth
- **Urdu**: Kaith
3.1.4 PARTS USED
Fruits, gum, leaves, bark and pulp are used traditionally.

3.1.5 PREVIOUS WORK
3.1.5.1 MEDICINAL USES AND BIOLOGICAL ACTIVITY

The plant is well known for its medicinal use. Various parts of wood apple have been utilized against ailments in ethnomedicine. The leaves are aromatic and carminative and are prescribed in indigestion and in bowel affictions of children. They are also used as astringent, in vomiting, hiccough and dysentery and are used traditionally in Ayurveda as antiemetic, aromatic, astringent, carminative, cardio tonic, expectorant, purgative, sudorifie, useful in anorexia, bronchitis, calculus, cardiac debility, cough, diarrhoea, gastropathy, hiccup and in vitiate conditions of vayu 5, 3, 7, 2, 8, 9.

The unripe fruits are sour, aromatic, astringent, constipating, alexipharming and are useful in diarrhea, pruritus and pharyngodynia, increases “vata”, “pita” and “kapha” and useful in whooping cough. The Hindus consider the unripe fruit to be a useful astringent in diarrhea and dysentery. It is used as a substitute for bael (Eagle marmelos) in treatment of diarrhea and dysentery. Ripe fruits are used as cardiotonic, tonic to liver and lungs, astringent and binding, diuretic and for strengthening the gums. The fruits are also beneficial in scurvy and sore throat. The pulp is applied externally for the bites of venous insects. Dry powder of the pulp is also used against diabetes 5, 7, 2, 8, 10. The bark is aromatic and is useful in vitiated conditions of pitta. It is occasionally prescribed for biliousness and useful in liver diseases 5, 3, 2, 11, 12, 13, 14, 9, 15.

Chemical constituents and antibacterial activity of the leaf essential oil of F. elephantum have also been studied which showed moderate activity against tested pathogens 16, 17. There are reports on the antimicrobial, antidiarrheal, hypolipidemic and hypoglycemic activity of the constituents extracted from leaves, fruits and bark of F. elephantum 18, 19, 20, 21, Pharmacognostical and phytochemical studies on the leaves of Feronia elephantum have also been
reported. The methanol extract of *Feronia elephantum* syn. *L. acidissima* possesses significant dose-dependant wound healing and anti-oxidant activities. The essential oil of *Feronia elephantum* Correa has been investigated for its antibacterial and antifungal activity against ten bacteria and ten fungi from which the oil exhibited strong to moderate activity against most of the test organisms. *Bacillus subtilis, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Shigella sp., Aspergillus niger, Rhizopus nodosus, Trichophyton rubrum 5S* and *Trichoderma viride* had remarkable susceptibility to the oil.

### 3.1.5.2 CHEMICAL COMPOSITION

The Phytochemical investigation of leaves of *F. elephantum* revealed the presence of methyl chavicol as the chief constituent in the essential oil. Methyl chavicol (68.3%), *trans*-anethole (4.7%), eugenol methyl ether (3.6%), anisaldehyde (4.4%), *para*-methoxy-cinnamic aldehyde (1.4%) and *para*-methoxy-cinnamic alcohol (1.5%) were reported as major constituents in the leaf essential oil of *F. elephantum* from Agra, India. Further, Ahmad, et al. (1989) identified methyl chavicol (27.20%), *trans*-anethole (10.94%), thymol (24.40%) and *p*-cymene-7-ol (7.30%) as major constituents from the leaves of *F. elephantum*.

Several flavonoids and coumarins were also reported from *F. limonia*. A new monoterpenoid furano coumarin lactone (fernoline) along with aurapten, marmesin, bergapten and xanthotoxin has been isolated from the roots of *F. elephantum*. Compounds isolated were stigmastriol from light petroleum extract, psoralen by the ether extract while orientin, vitexin and saponarin by ethyl acetate extract from the leaves of *F. elephantum*.

To the best of our knowledge, there are no reports on essential oil composition and antimicrobial activity of *F. elephantum* from Kumaun region. Therefore, the aim of the present investigation was to examine the essential oil composition and antimicrobial activity of the aerial parts of *F. elephantum* grown in Kumaun region (Uttarakhand, India) of Western Himalaya.
3.2 EXPERIMENTAL

3.2.1 PLANT COLLECTION AND IDENTIFICATION

The fresh aerial parts of *F. elephantum* were collected in the month of September from Udham Singh Nagar district (Uttarakhand, India). The plant was identified by Prof. Y.P.S. Pangtey, Botany Department, Kumaun University, Nainital and voucher specimens (BSD-15589) have been deposited in Botanical Survey of India, Dehradun for future reference.

3.2.2 ESSENTIAL OIL ANALYSIS

3.2.2.1 EXTRACTION OF ESSENTIAL OIL

The fresh leaves (2 kg) were subjected to steam distillation using a copper electric still, fitted with spiral glass condensers. The distillate was saturated with NaCl and extracted with *n*-hexane and dichloromethane. The organic phase was dried over anhydrous sodium sulfate and the solvent were distilled off in a rotary vacuum evaporator at 30°C and the percentage oil content was calculated on the basis of fresh weight of plant material. The oil yield was 0.4% (v/w).

3.2.2.2 THIN LAYER CHROMATOGRAPHY (TLC)

TLC experiment was done on silica gel coated plates. Spraying reagent used was anisaldehyde and iodine.

3.2.2.3 GAS CHROMATOGRAPHY ANALYSIS

GC analysis was performed in Perkin Elmer Auto System XL GC under following conditions.

- **Column**: EQUITY-5 (60m x 0.32mm x 0.25μm)
- **Carrier gas**: Hydrogen at 10 psi column head pressure
- **Detector**: Flame Ionization Detector
- **Injection temperature**: 280°C
- **Detector temperature**: 290°C
Initial oven temperature 70°C @ 3°C/min
Final oven temperature 290°C
Sample injection 0.05 µl Neat
Split ratio 1:30

3.2.2.4 GAS CHROMATOGRAPHY-MASS SPECTROMETRIC ANALYSIS

GC-MS was done on Autosystem XL GC coupled with Perkin Elmer Terbomass with following conditions.
Column EQUITY-5 (60m x 0.32mm x 0.25µm)
Carrier gas Helium at 10 psi column head pressure
Detector Flame Ionization Detector
Injection temperature 220°C
Ion source and Heater temperature 250°C
Initial oven temperature 70°C @ 3°C/min
Final oven temperature 290°C
Sample injection 0.05 µl Neat
Split ratio 1:50
Mass range 40-450 amu (WILEY and NIST libraries)

Sample components were identified by matching their mass spectra with those in WILEY and NIST, MS library search and by comparing with literature and GC retention indices (RI) 36.

3.2.2.5 ISOLATION OF COMPOUNDS

The fresh leaves oil of *F. elephantum* Correa, 5.0 mL was fractionated using column chromatography on a column packed with 100 g silica gel (230-400 mesh) in n-hexane. The compounds were eluted with n-hexane followed by n-hexane: Et₂O mixture gradually increasing the concentrations of Et₂O (5 to 20%) in n-hexane. The fractions collected were examined by TLC on silica gel G plates. The fractions with identical composition were mixed finally giving
useful fractions which were concentrated and further examined by TLC, IR spectroscopy and GC under isothermal and column temperature programmed conditions. There were subjected to repeated column chromatography to isolate two pure fractions Scheme 3.1.

Flow sheet of CC of essential oil of *Feronia elephantum* Correa.

Scheme 3.1 Isolation of compounds from *Feronia elephantum* Correa
3.3 RESULTS AND DISCUSSION

3.3.1 ESSENTIAL OIL COMPOSITION

The identified constituents of the oil are listed in Table 3.1 in order of their elution in EQUITY-5 column. The GC (Figure 3.1) and GC-MS analysis of *Feronia elephantum* essential oil showed the presence of 24 compounds, of which 18 compounds were identified, representing 99.39% of the total oil composition. The earlier report showed the presence of methyl chavicol (27.20%) *trans*-anethole (10.94%), thymol (24.40%) and *p*-cymene-7-ol (7.30%) as major constituents. On the contrary, our results differ considerably having *trans*-anethole (57.73%) and methyl chavicol (37.48%) as the major compounds with complete absence of thymol and *p*-cymene-7-ol. The other constituents identified were *cis*-anethole, *p*-anisaldehyde, (*E*)-jasmine, methyl eugenol, β-caryophyllene, linalool and (*E*)-methyl isoeugenol.

A major part of the essential oil of *F. elephantum* was dominated by oxygenated monoterpenes (98.84%). The presence of phenyl derivatives viz. *trans*-anethole, methyl chavicol, *cis*-anethole, methyl eugenol and (*E*)-methyl isoeugenol in such high percentage makes the oil strongly odoriferous for various pharmaceutical preparations. Pharmaceutical drugs derived from or related to anethole include anisyldithiolthione, *anethole dithione* (ADT) and *anethole trithione* (ATT). Chronic treatment with trithio-β-p-methoxyphenylpropene (anethole trithione; ATT) increased the salivary secretion.
Figure 3.1 Gas Chromatogram of the essential oil of *Feronia elephantum* Correa.
3.3.2 CHARACTERIZATION OF CONSTITUENTS

3.3.2.1 PHYSICOCHEMICAL DATA OF COMPOUND F#01

Physicochemical data

IR \( \nu_{\text{max}} \) cm\(^{-1} \) (Figure 3.2): 3060, 3049, 2950, 1642, 1498, 1247, 1040, 909, 784, 692.

EIMS: 148 (M\(^+\)), 133, 121, 105, 91, 77, 63, 51, 41.

\(^1\)H NMR (300MHz, CDCl\(_3\)-TMS) \( \delta \) ppm (Figure 3.3)

3.56 (d, 2H), 3.73 (s, 3H), 5.10 (dd, 2H), 6.06 (m, 1H), 6.80 (d, 2H), 7.22 (d, 2H)

\(^{13}\)C NMR (75MHz, CDCl\(_3\)-TMS) \( \delta \) ppm (Figure 3.4)

39.90 (t, C\(_6\)), 55.26 (q, C\(_7\)), 113.89 (d, C\(_{2,6}\)), 115.54 (t, C\(_{10}\)), 126.86 (d, C\(_{3,5}\)), 136.23 (s, C\(_4\)), 137.84 (d, C\(_9\)), 158.56 (s, C\(_1\))

The compound FE#1 isolated was dark yellow liquid. The EIMS of the compound showed a molecular ion peak at m/z 148 corresponding to the molecular formula C\(_{10}\)H\(_{12}\)O. \(^1\)H NMR representing six types of non equivalent protons. The IR spectrum showed strong peak at 1247 cm\(^{-1}\) confirms the presence of (ARYL-O-C) aryl ether. The spectrum showed a doublet signal at \( \delta \) 3.56 a methylene group attached to an aromatic ring. A singlet at \( \delta \) 3.73 corresponds to the protons of methoxy group which possess no adjacent proton. The signal at \( \delta \) 5.10 refers to the overlap of 2 protons (dd) of the olefinic protons. Both protons are attached to the same carbon, though, they are not equivalent due to the restricted rotation in C=C. Both proton couple with each other and the yield overlapping double doublet (dd) signals. The signal at \( \delta \) 6.06 a multiplet signal corresponds to olifinic proton attached to substituted carbon. The protons at 6.80 (d, 2H) and 7.22 (d, 2H) is due to presence of aromatic protons. The \(^{13}\)C-NMR of the compound showed a total of ten carbons and their multiplicity assignment marked the presence of one CH\(_3\), Two CH\(_2\), five CH and two quaternary carbon atoms. One allylic system has been confirmed by carbon resonance at \( \delta \) 115.54, 137.84 and 39.91. On the basis of the above spectral data FE#01 was characterized as methyl chavicol, a monoterpenoid 39.
Methyl chavicol
Figure 3.2 IR Spectrum of FE #1
Figure 3.3 $^1$H NMR Spectrum of FE #1
Figure 3.4: $^{13}$C NMR Spectrum of FE #1
3.3.2.2 CHARACTERIZATION OF CONSTITUENTS

Physicochemical Data of Compound F#02

IR $\upsilon_{\text{max}}$ cm$^{-1}$ (Figure 3.5): 3023, 3003, 2958, 1698, 1261, 1036, 965, 840, 756.

EIMS: 148(M$^+$), 133, 117, 105, 91, 77, 63, 51.

$^1$H-NMR (300MHz, CDCl$_3$-TMS) $\delta$ ppm (Figure 3.6)

1.82 (dd, 3H), 6.06 (dq, 1H), 6.32 (dq, 1H), 6.80 (d, 1H), 7.22 (d, 1H), 3.73 (s, 3H)

$^{13}$C-NMR (75MHz, CDCl$_3$-TMS) $\delta$ ppm (Figure 3.7)

18.31 (q, C$_{10}$), 55.26 (q, C$_7$), 113.89 (d, C$_2$, C$_6$), 123.48 (d, C$_9$), 126.86 (d, C$_3$, C$_5$), 130.32 (d, C$_8$), 130.81 (s, C$_4$), 158.56 (s, C$_1$)

The compound F#02 isolated was a light yellow liquid. The EIMS of the compound showed a molecular ion peak at m/z 148 corresponding to the molecular formula C$_{10}$H$_{12}$O. Strong peak at 1261 cm$^{-1}$ in IR spectrum confirms the presence of C-O-C starching of aryl ether. The singlet of three protons at $\delta$3.73 in $^1$H NMR showed the presence of $-$OCH$_3$ group. The peaks at 965 cm$^{-1}$ due to out-of-plane banding confirmed the presence of t-alkene double bond. Four aromatic protons, two at $\delta$ 6.80 and two at 7.22 supported that a para-disubstituted aromatic ring was present, as did a strong IR absorption band at 840 cm$^{-1}$ due to an out-of-plane C-H banding. $^{13}$C-NMR of the compound showed two aromatic CH resonances at $\delta$ 113.79 and 126.78 showing symmetrically substituted aromatic ring. The two one proton multiplets were present in the proton spectrum, one centered at $\delta$ 6.06 and the other centered at $\delta$ 6.32, supported the presence of disubstituted double bond. The $^{13}$C-NMR of the compound showed a total of ten carbons and their multiplicity assignment marked the presence of two CH$_3$, six CH and two quaternary carbon atoms. On the basis of the above spectral data FE#02 was characterized as trans-anethole, a monoterpenoid.
trans-anethole
**Figure 3.5** IR Spectrum of FE #2
Figure 3.6 $^1$H NMR Spectrum of FE #2
Figure 3.7 $^{13}$C NMR Spectrum of FE #2
3.4 CONCLUSIONS

The above results suggested that *F. elephantum* was found to contain 24 compounds of which 18 compounds were identified. *Trans*-anethole (57.73%) and methyl chavicol (37.48%) were the major compounds along with *cis*-anethole, *p*-anisaldehyde, *(E)*-jasmone, methyl eugenol, *β*-caryophyllene, linalool and *(E)*-methyl isoeugenol as minor constituents. This is the first report on the essential oil composition of *F. elephantum* collected from Kumaun region (Uttarakhand, India) of Western Himalaya. The presence of *trans*-anethole (57.73%) and methyl chavicol (37.48%) in such a high amount makes the plant useful for commercial utilization for cosmetics, pharmaceutical and other allied industries.
Table 3.1: Essential oil composition of the aerial parts of *F. elephantum*

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<th>Peak No.</th>
<th>Compounds</th>
<th>Group of compound</th>
<th>RI</th>
<th>% (FID)</th>
<th>Mode of Identification</th>
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<tr>
<td>2</td>
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Mode of identification: (a) Retention Index on Rtx-5 column MS (b) MS (GC-MS), (c) NMR (1H- and 13C-NMR), t = trace (<0.10%).
3.5 REFERENCES


34. Talapatra, S. K., Chaudhary, M. K. and


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