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EVALUATION OF PHYTOCONSTITUENTS OF *FERONIA LIMONIA* AND ITS *IN VITRO* CYTOTOXICITY

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Abstract*: For the continuation of phytochemical research and isolation of active constituents, the present investigation evaluated the preliminary phytochemical screening and in vitro cytotoxicity of ethanol extract of the tree Feronia limonia which belongs to family Rutaceae and have many traditional uses. Phytochemical screening revealed the presence of flavonoids, steroids, coumarins, phenolics and terphenoids. The ethanol extract of F. limonia was subjected to GC-MS analysis, a total of 17 compounds were identified and the major compounds of F. limonia were 6-methyl-1-(1-naphthyl)dihydrouracil (9.12), 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl (5.99), Eicosane (5.08), Nonadecane (5.52), Pentadecane, 2,6,10,14-tetramethyl (5.05), Eicosane (5.08) and other compounds. Invitro cytotoxicity was performed using MOLT-3 cancer line by MTT assay, which showed IC_{50} value of 63.24µl. The ethanol extract of this plant has significant cytotoxicity against tested cell line.*

Keywords: *F. limonia, MOLT-3, coumarins, flavonoids and MTT assay.*

I.Introduction

Feronia limonia belongs to the family Rutaceae has many synonyms include *Feronia elephantum* Correa *and Limonia acidissima* Linn. is a small tree found in India and South east Asia. The plant has a long history of traditional usage, tocure asthma, tumors, opthalmia, leucorrhoea, scurvy and sore throat. The fruit pulp is sour, sweet, edible, stimulant and astringent. The bark is aromatic, having cool sensation and is useful in vitiated conditions of pitta. The bark is occasionally prescribed for biliousness and useful in liver disease, The fruit is used in India as a liver and cardiac tonic, and when unripe, as an astringent means of halting diarrhoea and dysentery and effective treatment for hiccough, sore throat and diseases of the gums. The pulp is poulticed onto bites and stings of venomous insects, as is the powdered rind. Juice of young leaves is mixed with milk and sugar candy and given as a remedy for biliousness and intestinal troubles of children. The powdered gum, mixed with honey, is given to overcome dysentery and diarrhea in children. Oil derived from the crushed leaves is applied onto the itch and the leaf decoction is given to children as an aid to digestion. Leaves, bark, roots and fruit pulp are all used against snakebite. The bark is chewed with that of Barringtonia and applied on venomous wounds (Nandkarni, 1976 ; Kirtikar and Basu, 1998; Dymock *et al*., 2005).

The different parts of the plant have been investigated by several workers and found to contain coumarins, furanocoumarins, lignans, alkaloids, steroids and flavonoids. The unripe fruits contain Stigmasterol (Anonymous). The pharmacological studies, like antioxidant, antibacterial Activity (Garg, 2001; Momin *et al*., 2013; Nithya and Saraswathi, 2010; Mehta *et al*., 1983; Panda), anti tumor Activity (Saima *et al*, .2000) cytotoxicity (Sampandan and David., 2014; Sadia Shermin *et al*., 2012; Hossain, 2013), anti inflammatory (Ahamed *et al*., 2008) and hepatoprotective (Illango and Chitra, 2009)

Cancer is a dangerous disease producing abnormal cell growth which affects various parts of the body. Like different types of cancer, cancer curing synthetic drugs also available with side effects. The plant based drugs are tested against cancer since from ancient times, in this view every medicinal plant is tested for its anticancer potential. Our present study focused to evaluate preliminary screening and it's *in vitro* cytotoxic study of ethanol extract of *F.limonia* using MOLT-3 cancer cell line by MTT assay.

II. Materials and Methods

Plant material

Feronia limonia under the plant family Rutaceae was collected from Pollachi, Tamil Nadu, South India. The plant sample was authenticated by Department of Botany and the voucher specimens have been kept for further reference.

Extraction Process

The leaves of 500g of *F.limonia* were dried in shade for 10 days and tightly packed for further process. Air dried leaves of *F.limonia* was chopped into small pieces. The coarse material was subjected to maceration and Soxhlet extraction by using different solvents used based on their increasing order of polarity, i.e. petroleum ether. The defatted plant leaves were again extracted with ethanol using Soxhelt apparatus. The extract was subjected to vacuum distillation and was concentrated to yield brownish residues of 80g.

Preliminary phytochemical screening

Ethanol extracts of *F.limonia* were subjected to qualitative chemical analysis to identify the nature of phytochemical constituents present in it. The test for steroids, phenol, tannis, fatty acid, alkaloids, flavonoids, saponins and coumarins was performed.

GC-MS analysis

GC-MS analysis of the phytochemical of *F.limonia* carried out using thermo GC – trace ultra-version: 5.0 coupled with thermo MS DSQ II instrument. The compounds were separated on DB-35, MS capillary DSQ II instrument. The MS capillary standard non-polar column (0.25mm), film thickness 0.25µm. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 70˚C and held for 2 minutes and the temperature of the oven was raised to 260˚C for 10 minutes, raised 6˚C per minute and the final temperature was 300˚C for 10 minutes.

Identification of Phytoconstituents

The components were identified by comparison of their mass spectra with those of the National Institute of Science and technology (NIST) mass spectral library version 2.0d, as well as on their comparison of their retention time either with those of authentic compounds or with their literature values.

***In vitro* cytotoxicity by MTT assay**

The *in vitro* anticancer activity of ethanol extract of *F.limonia* leaves were tested against the MOLT-3 cancer cell line. MOLT-3 cancer cell is the adult T acute lymphoblastic leukemia cell line. In MTT assay the cell viability of cancer cells after incubation with different concentration of the leaf extract was examined. 3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 hours of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37˚C for 4 hours. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570nm using micro plate reader (T.Mosmann., 1983). The % cell inhibition was determined using the formula

% cell inhibition = 100 – [Abs (sample)/Abs (control)] * 100

IC_{50}value was determined by Nonlinear regression graph was plotted between % cell inhibition and log_{10}concentration.

III. RESULT AND DISCUSSION

Preliminary Phytochemical Screening

Ethanol extracts of *F. limonia* were subjected to qualitative chemical analysis to identify the nature of phytochemical constituent present in it. alkaloids, terpenoids ,steroids, phenolics and excess amount of coumarins were present in the ethanol extract of leaves of *F. limonia* which was shown in table.1.

Table 1: Phytochemical constituents of *F. limonia*

S.No	Phytoconstituents	Phytoconstituents
1	Fatty acid	-
2	Alkaloids	+
3	terpenoids	+
4	Flavonoids	+
5	Steriods	+
6	Saponins	-
7	carbohydrate	-
8	Phenolics	+
9	Comarins	+

+ presence of phytochemicals, - Absence of phytochemicals

GC-MS ANALYSIS

The ethanol extract of *F. limonia* was subjected to GC-MS analysis, a total of 17 compounds were identified and

was given in table2. and figure2.The major compounds of F. limonia were 6-methyl-1-(1-naphthyl)dihydrouracil (9.12), 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl (5.99), Eicosane (5.08), Nonadecane (5.52), Pentadecane, 2,6,10,14-tetramethyl (5.05), Eicosane (5.08), -Caryophyllene oxide (4.82), Hexadecane (3.95), Myrtenol (3.44), á-Cubebene (1.63), GERMACRENE-D (2.13), 1-Hexadecene (1.8), other compounds were Tricosane (1.29), docosane (1.30), noncosane (1.14) etc,.

Figure.1.GC-MS chromatogram of ethanol extract of *F.limonia*

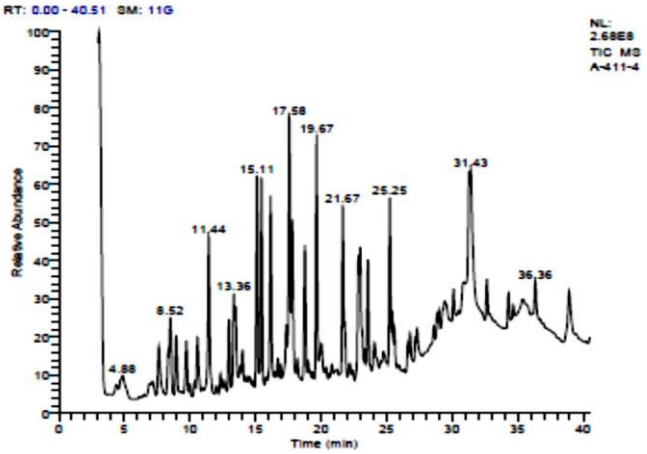


Table.2 GC-MS Analysis of ethanol extract of *F.limonia*

S.NO	NAME OF THE COMPOUND	RETENTION TIME (min)	(%) of the compound
1	Iso-pinocampheol	4.88	1.25
2	1,3-O-Benzylidene-1,3-dihydroxy-4-	7.63	1.78
3	Myrtenol	8.52	3.44
4	1-Hexadecene	9.74	1.8
5	Germacrene-D	10.53	2.13
6	á-Cubebene	12.98	1.63
7	Hexadecane	13.36	3.95
8	Pentadecane, 2,6,10,14-tetramethyl	15.11	5.05
9	-Caryophyllene oxide	16.18	4.82
10	6-methyl-1-(1-naphthyl)dihydrouracil	17.58	9.17
11	Nonadecane	19.67	5.52
12	3-(4-Methyl-1,3-cyclohexadienyl)butanoic acid	20.04	1.47
13	Eicosane	21.67	5.08
14	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl	25.25	5.99
15	Tricosane	27.35	1.29
16	Docosane	32.67	1.30
17	Nonacosane	36.36	1.14

In vitro Cytotoxicity

The present study examined *in vitro* cytotoxicity of ethanolic extract of *F.limonia* leaves against MOLT-3 cells (adult T acute lymphoblastic leukemia cell line) by MTT assay which measures the % inhibition of cancer cells. The ethanol extract of leaves of *F.limonia* was diluted in different concentrations and the result, was shown in the Table (5). The extract was diluted to 5µl, 25µl, 50µl, 75µl, 100µl. The viability of cancer cell after incubation was given in the figure (3). The ethanol leaves extract of *F.limonia* showed moderate to severe cytotoxicity on MOLT-3 cells in dose dependent pattern. The IC_{50} value of cell viability was 63.24µl. The result revealed that the ethanol extract of *F.limonia* showed potent *in vitro* cytotoxicity against the MOLT-3 cancer cell line. The obtained results are comparable with results of (Shermin *et al*., 2012). There are few reports only available for *in vitro* cytotoxicity of this plant.

Figure 3. – Plot of % cell inhibition of ethanol extract of *F.limonia* leaves.

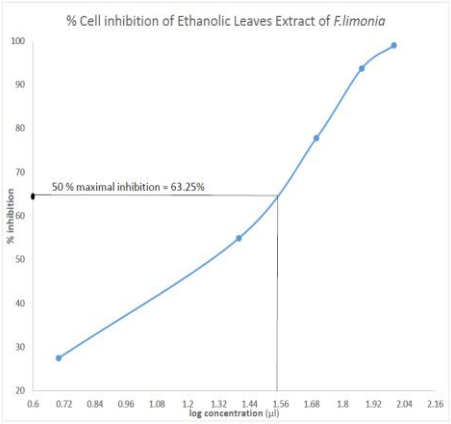
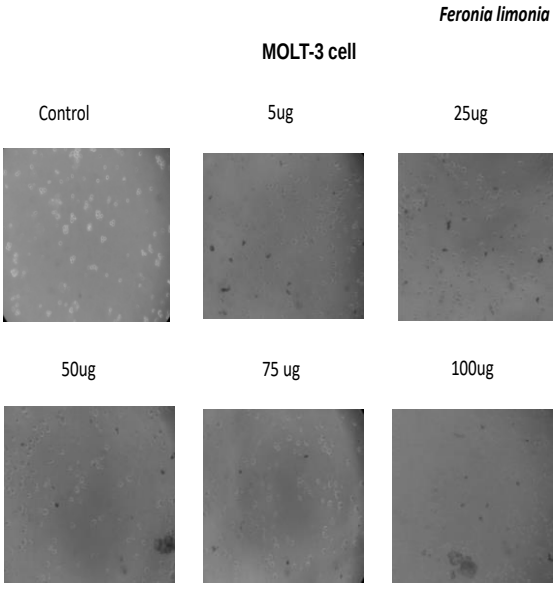


Figure 4. The cell viability of MOLT-3 cancer cell line of ethanol extract of leaves of *F.limonia*



IV. Conclusion

The present investigation revealed that the ethanol extract of *F.limonia* showed the presence of many active phytochemicals like, alkaloids, flavonoids, coumarins and steroids . *In vitro* cytoxicity study using MOLT-3

cancer cell line by MTT assay was performed and have the IC_{50} of 63.24 µl, which showed the extract has significant cytotoxicity against cancer cells.

NO Conflict of interests

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