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FTIR, phytochemical and antibacterial activity of different extract of *Feronia Limonia* leaves

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Abstract
The present study deals with the phytochemical examination of therapeutic importance of leaves extract of *feronia limonia*is an important medicinal plant. Phytochemical analysis of the five extracts prepared from *feronia limonia* leaves revealed the presence of carbohydrate, alkaloids, glycoside, saponin, phytosterol, phenol, tannin, flavonoid, protein and amino acid. The FT-IR spectrum confirmed the presence of characteristic functional group.. The aqueous, ethanol, chloroform, acetoneand petroleum ether extract of *feronia limonia* leaves were tested against Staphylococcus aureus, Salomonella typhimurium , Proteus vulgaris, Pseudomonas aeruginosa and Bacillus megaterium by the cup-plate method.

Keywords: *feronia limonia* leaves, phytochemical screening, FT-IR, antibacterial activity

1. Introduction
Nearly all the medicinal plants available in the world have great potential sources for discovery as well as protection of new drugs of benefit to mankind. Now a daya lot of approaches available to reach for new biologically active ingredients in the medicinal plants for the preparation of safe drugs. Scientifically many works have been expended to evaluate and discover new antioxidant, antimicrobial and antifungal ingredients from different kinds of natural sources like soil, microorganisms, animals and plants. Different types of folk medicine or herbal medicine are among the most important resources. Check and need to systematic screening of these available traditional herbs may result in the discovery of novel effective bioactive compounds for the formulation of drugs [1]. Its leaves are commonly used as carminative, purgative, Bronchitis, Diarrhoea, Expectorant and cardiotonic. The juice of green leaves of feronia limonia is used for intestinal trouble of children. The Feronia limonias fruits are used for disease like tumors, Asthma, wounds, cardiac, Debility and Hepatitis. Feromia limonia belongs to family Rutaceae, is commonly known as kaith or wood apple and is widely distriduted in most tropical and subtropical countries [2]. The feronia limonia is native and comman in India, Shrilanka, China and Indonesia [3]. Feronia limonia as a whole or its parts such as unriped fruit, riped fruit, root bark, trunk, gum and leaves have a broad spectrum of traditionally established therapeutic properties [4] and widely used in several Ayurvedic preparation like panch kapittha[5]and kapitthaashtaka churna [6]. Leaves of feronia limonia showed anthelminitic activity [7]. The main chemical constituents of feronia limonia were flavonoids, saponins and tannin [8-11].
The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raise serious concern of health delivery and accessibility due to untreatable bacterial infections. There is therefore the needed urgently to the search for new antimicrobial drug. Plants are important source of potentially useful structures for the development of new chemotherapeutic agents [12]. To the best of our knowledge, there is no record of work on the phytochemical screening, FT-IR analysis and antibacterial activity of the *feronia limonia* leaves. Therefore, the present study was carried out to evaluate the phytochemical screening, FT- IR analysis and antibacterial activity of the *feronia limonia* leaves.

2. Materials and Methods
2.1 Collection of medicinal plants
The fresh leaves of *Feronialimonia* collected from Mahadeodara, District Beed.The fresh leaves were dried under shade, powdered and pass through 40 mesh sieve and stored in closed bottle for further use. The powder was extracted with different solvent such as water, ethanol, chloroform, acetone, petroleum ether by Soxhlet apparatus. Phytochemical analysis were carried out for all the extract as per the standard methods [13].

3. Screening of antibacterial activity
3.1 Bacterial Strains
The test organisms were purchased from NCIM, NCL Pune. The organisms were sub-cultured in the media specified. The organisms, their ATCC code, media in which they are sub-cultured are given in Table No.1. Bacteria were incubated at 37 ^{0}C in incubator for 24 hrs. They were further stored at 4 ^{0}C in the refrigerator to maintain stock culture. Microorganisms with their ATCC Codes and media used for subculture are as follows [14].

Table 1: ATCC code and media used for development of micro-organism.

Sr. No.	Name of microorganism	ATCC Code	Media
1	*Salmonella typhimurium*	2501	Nutrient Agar
2	*Bacillus megeterium*	2087	Nutrient Agar
3	*Pseudomonas aeruginosa*	2200	Nutrient Agar
4	*Staphylococcus aureus*	2079	Nutrient Agar
5	*Proteus vulgaris*	2027	Nutrient Agar

4. Result and Discussion

Phytochemical analysis of *feronia limonia* leaves shows presence of saponins, protein & amino acid in aqueous extract. Whereas phenol, tannin, flavanoids are present in other extract. Phytomedicine has been used for the treatment of chronic diseases. Phenolic compounds have been reported to be potential free radical scavengers. The plants rich in tannins have significant activity in cancer prevention and are used in treating intestinal disorders. Flavonoids are known to possess a wide range of biological activities such as antioxidant, antimicrobial, anti-inflammatory and anticancer activities[15]. In five extract ethanol extract shows presence of carbohydrates, alkaloid, phenol, tannin, flavanoids which is represented in Table.2.Antibacterial activity only shown by aqueous, ethanol and acetone extract represented in Table.3. IR Peak values of *feronia limonia* for different extract represented in Table.4.

In the aqueous extract of *Feromia limonia* consists of characteristics peak of 2929 cm^{-1} due to C-H stretching associated with aromatic ring. The bands at 1590 cm^{-1} & 1392 cm^{-1} due to asymmetric and symmetrical stretching of $-NO_2$ group respectively. The peak at 3276 cm^{-1} & 2929 cm^{-1} is due to N-H stretching and C-H stretching of alkyl group respectively. In ethanolic extract the peak at 3340 cm^{-1} and 2849 cm^{-1} are due to O-H stretching , C-H stretching of –CHO group. The peaks at 2917 cm^{-1} & 1710 cm^{-1} are due to C-H stretching and C=O stretching. The band found at 1231 cm^{-1} due to C-O-C asymmetric stretching of aromatic ether. The band at 1461 cm^{-1} confirmed presence of benzene ring. The chloroform extract of *Feromia limonia* shows characteristic peak at 3300 cm^{-1} due to ≡C-H group. The peaks at 2917 cm^{-1} & 1711 cm^{-1} due to C-H stretching and C=O stretching. The peak obtained at 1375 cm^{-1} due to $-NO_2$ group. The C-O stretching band obtained at 1061 cm^{-1}. Acetone extract shows characteristics peak at 2916 cm^{-1} & 1462 cm^{-1} due to C-H stretching of aromatic benzene ring. 2848 cm^{-1} & 1709 cm^{-1} due to Ar-H and C=O group. The band at 1376 cm^{-1} & 719 cm^{-1} due to $-NO_2$ group and C-Cl mono substituted benzene ring. Petroleum ether extracts shows presence of C-H stretching and C-O stretching due to 2916 cm^{-1} and 1710 cm^{-1}. The peak found at 719 cm^{-1} and 729 cm^{-1} due to C-Cl stretching. IR Peak values of *feronia limonia* for different extract represented in Table.3.

Table 2: Phytochemical Constituents of*feronia limonia* leaves.

Sr. no	Chemical constituents	Aqueous extract	Ethanol extract	Chloroform extract	Acetone extract	Petroleum ether extract
1	Carbohydrate	-	+	-	-	-
2	Glycoside	-	-	-	-	+
3	Alkaloid	-	+	-	-	+
4	Phytosterols	-	-	+	-	+
5	Saponins	+	+	-	-	-
6	Phenol	-	+	+	+	+
7	Tannin	-	+	+	+	+
8	Flavanoids	-	+	+	+	+
9	Protein & Amino acid	+	+	-	-	-

+ Present, - Negative

Table 3: Antibacterial activity of *feronialimonia*leaves in different solvent.

Sr. No	Name of organism	Aqueous extract mm	Ethanol extract mm	Chloroform extract mm	Acetone extract mm	Petroleum ether extract mm
1	*Staphylococcus aureus*	8	2	-	-	-
2	*Salomonellatyphimurium*	-	-	-	1	-
3	*Proteus vulgaris*	5	4	-	-	-
4	*Pseudomonas aeruginosa*	4	2	-	-	-
5	*B.negaterium*	10	-	-	-	-

Table 4: IR Peak value of *feronia limonia* for different extract

Sr. No.	*Extracts*	*I R Observed peaks (cm^{-1})*
1	Water	3276 , 2929, 1590, 1392, 1258, 1074
2	Ethanol	3340, 2917,2840, 1710, 1614, 1514, 1375, 1461, 1231, 1047
3	Chloroform	3300,2917, 2849, 1711, 1607, 1375, 1242, 1061
4	Acetone	2916, 2848, 1709, 1462, 1376, 1083, 983, 719.
5	Petroleum ether	2916, 2848, 1710, 1462, 1376, 1082, 983, 729, 719.

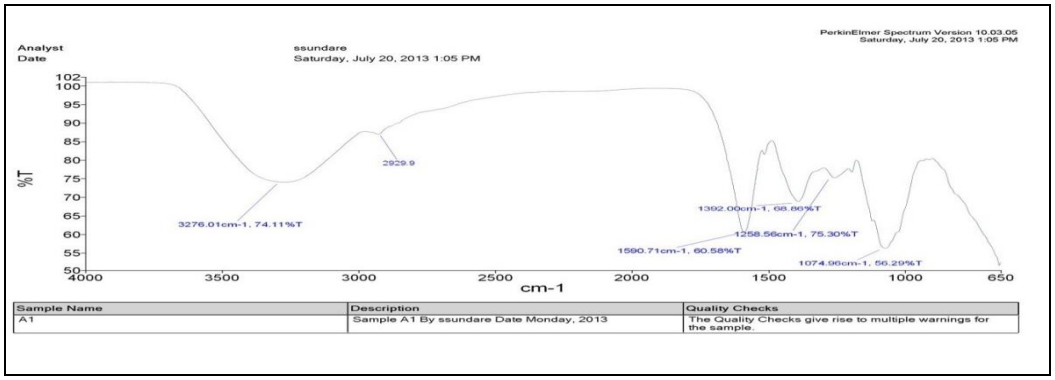

Fig 1: I R of *Feronia limonia* in aqueous extract

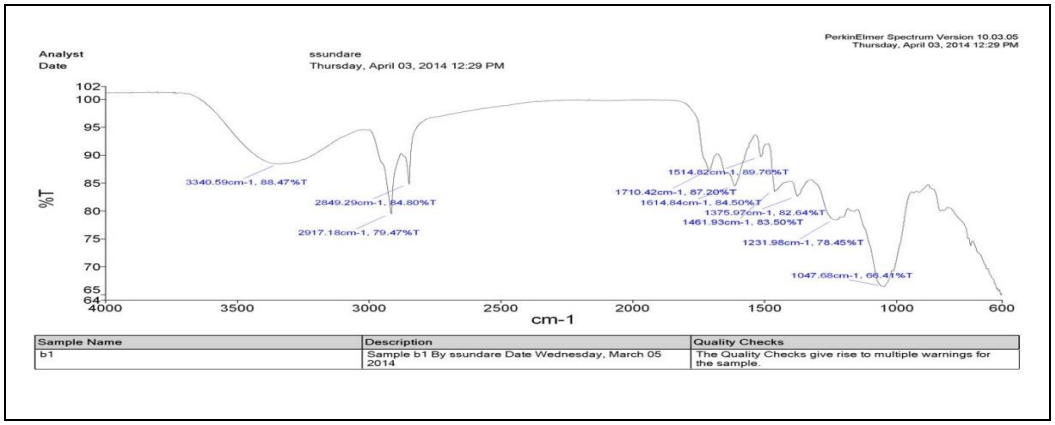

Fig 2: I R of *Feronia limonia* ethanol extract.

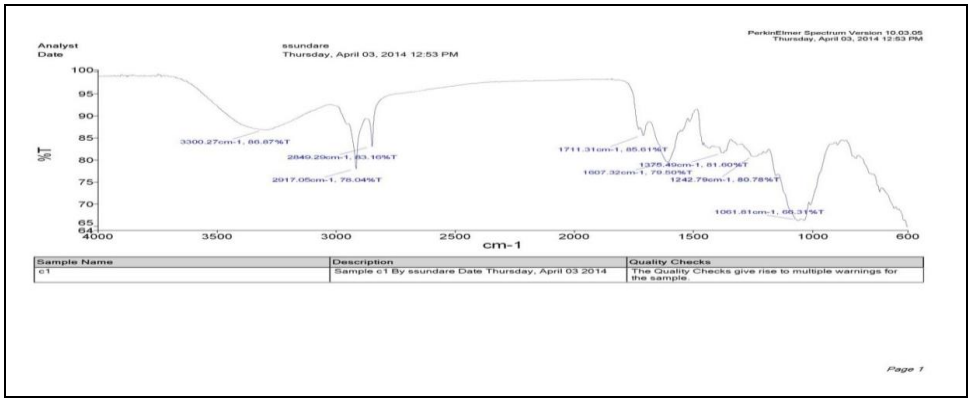

Fig 3: IR of *Feronia limonia* in chloroform extract.

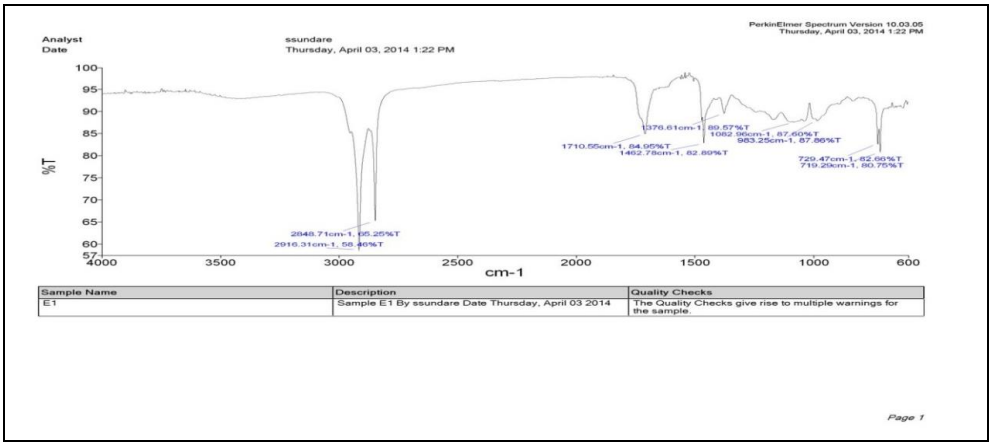

Fig 4: I R of *Feronia limonia* in acetone extract.

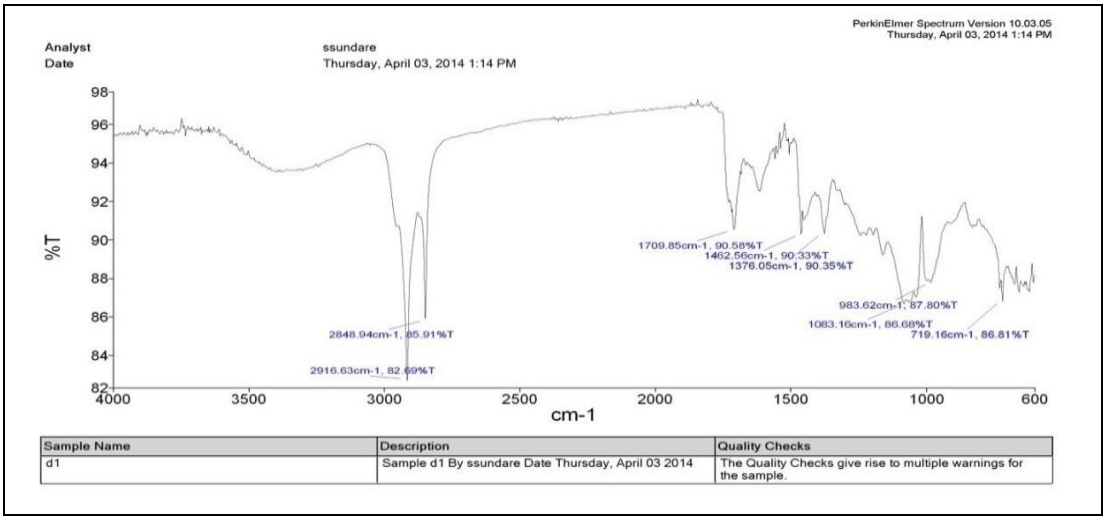

Fig 5: I R of *Feronia limonia* in petroleum ether extract.

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