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Research Article

FOURIER TRANSFORM INFRARED SPECTROSCOPY SPECTROSCOPIC STUDIES IN *EMBELIA RIBES* BURM. F.: A VULNERABLE MEDICINAL PLANT

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ABSTRACT

Objective: The present study was aimed to identify the functional group present in the crude powder and various solvent extracts of *Embelia ribes* Burm. f. stem, leaves, and berries through Fourier transform infrared (FTIR) spectroscopy.

Methods: Different plant parts of *E. ribes* were collected shade dried, powdered, and extracted in methanol, ethanol, and petroleum ether. These extracts were used to detect the characteristic peak values and their functional groups using FTIR method on a OMNI sampler attenuated total reflectance accessory on a JASCO FTIR spectrophotometer (FTIR-4600).

Results: The crude powder of *E. ribes* leaves, stem, and berries FTIR analysis confirmed the presence of amino acids, amide, alkanes, carboxylic acids, alcohols, esters, ethers, aromatics, aliphatic amines, phenols, aldehyde, ketones, fluorides, halogen, alkyl halides, and nitro compound. The dry methanolic and ethanolic extracts of *E. ribes* leaves, stem, and berries FTIR analysis results proved the presence of alcohols, p-substituted alcohols or phenols, phenols, alkanes, alkynes, alkenes, aldehyde, ester, ether, aliphatic amines, carboxylic acids, aromatics, ketones, disulphide, alkyl halides, halogen, and nitro compounds, whereas dry petroleum ether extract shown the presence of amide, alkanes, carboxylic acids, alcohols, p-substituted alcohols or phenols, esters, aromatics, aldehyde, ketones, aryl disulphide, aliphatic amines, aliphatic compound, alkyl halides, and nitro compounds, respectively.

Conclusion: The results of the present study produced the FTIR spectrum profile for the vulnerable medicinally important plant *E. ribes* Burm. f.

Keywords: *Embelia ribes* Burm. f., Fourier transform infrared spectroscopy, Spectroscopy, Functional groups.

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INTRODUCTION

Fourier transform infrared (FTIR) spectrometry measures the vibrations of bonds within chemical functional groups and generates a spectrum that can be regarded as a biochemical or metabolic "fingerprint" of the sample. By attaining IR spectra from plant samples, it might possible to detect the minor changes of primary and secondary metabolites [1,2]. At present, particularly in phytochemistry, FTIR has been exercised to identify the concrete structure of certain plant secondary metabolites [3,4].

FTIR is one of the most widely used methods to identify the chemical constituents and elucidate the compound structures to propose in medicinal purposes [5,6]. Previous researchers carried out the FTIR to notice the minor changes of primary and secondary metabolites [1,2] and to recognize the concrete structure of certain plant secondary metabolites [7]. The characteristics functional groups are responsible for the medicinal properties of plant are confirmed by FTIR analysis [8] *Embelia ribes* Burm. f. locally known as false black pepper or vidanga belongs to family *Myrsinaceae*. It is an important medicinal dioecious woody climber, sparsely distributed in the evergreen to moist deciduous forests of the Western Ghats [9]. It is listed in the "priority species list" for cultivation by the National Medicinal Plant Board and the Maharashtra State Horticulture and Medicinal Plant Board [10]. It is used as an ingredient in about 75 traditional ayurvedic drug formulations [11]. The fruit of the *E. ribes* Burm. f. plant has been used to treat fever, inflammatory diseases, and a variety of gastrointestinal ailments for thousands of years [12]. More than four decades ago, the active component from this plant was isolated and named embelin [13] and later synthesizes [14]. Embelin has been shown to have antitumor, anti-inflammatory, and analgesic properties [15]. From pharmaceutical point of view, *E. ribes* contains embelin, fatty ingredients, quercitol, alkaloid christembine, tannins, and volatile oils.

FTIR provides biochemical profiles containing overlapping signals from a majority of the compounds that are present when whole cells are surely analyzed [16]. In the present investigation, crude powder and solvent extracts of root, stem, and berries of *E. ribes* were analyzed. With this background, the present study was aimed to report the main functional components of the present in leaves, stem, and berries of *E. ribes* using Fourier transform infrared spectroscopy (FTIR) spectrometry.

METHODS

Collection and processing of plant material

Plant material of *E. ribes* was collected from Devimane Ghats, Tal-Sirsi, District - Uttara Kannada, Karnataka, India (GPS-N 14°31.278', E 074°32.849'). The plant materials were identified at the Department of Botany, Shivaji University, Kolhapur. Voucher specimens were prepared and deposited in the herbarium of the Department of Botany, Shivaji University, Kolhapur. The leaves, stem, and berries were separately shade dried, cut into small pieces, and fine powder was prepared by grinding. Powdered material was stored in an airtight container for further use.

Preparation of extract

The powdered plant parts (leaves, stem, and berries) 10 g each were extracted in 100 ml of methanol, ethanol, and petroleum ether with continuous shaking on mechanical shaker for 24/hrs at room temperature. The extracts were then filtered through Whatmann No. 1 filter paper and stored in airtight vials for refrigeration. Stored extract was used for further analysis.

FTIR spectroscopic analysis

All spectra were obtained with the aid of OMNI sampler attenuated total reflectance accessory on a JASCO FTIR spectrophotometer (FTIR-4600) followed by previous methods with some modifications. A dry powder

of leaves, stem, and berries was encapsulated in KBr pellet, to prepare translucent sample discs. Small amount of each plant part sample extract of different solvents was, respectively, placed directly on the germanium piece of the infrared spectrometer with constant pressure applied. Data of infrared absorbance were collected over the wave number ranged from 4000/cm to 650/cm. The reference spectra were acquired from the cleaned blank crystal before the presentation of each sample replicate. All spectra were collected with a resolution of 4.0-1.0 cm and to improve the signal-to-noise ratio. Samples were run in triplicates. The FTIR spectrum of all samples was analyzed on the basis of peak values in the region of infrared radiation.

RESULTS AND DISCUSSION

The FTIR spectrum was used to identify the functional groups of the active components in plant samples based on the peak value in the region of infrared radiation [12]. The crude powder and solvent extract of stem, leaves, and berries of *E. ribes* gave the following characteristic absorption peaks as represented in Figs. 1-4 and Tables 1-4. The FTIR spectroscopy is proved to be a reliable and sensitive method for detection of biomolecular composition [13]. The absorption spectra of crude powder of *E. ribes* stem, leaves, and berries are shown in Fig. 1 and Table 1. The results of *E. ribes* crude stem powder FTIR analysis confirmed the presence of phenol at peak of 3775.23/cm. The peak at 3305.54/cm represents the presence of amine and amide as functional group. The peak at 2918.20 and 2854.03/cm confirms the presence of alkanes.

The peak at 1733.78/cmrepresents the presence of aldehyde and ketone. The peak at 1605.32, 1510.87, and 1433.68/cm indicated the presence of amino acid, nitro compound, and aromatic functional group, respectively. The peak at 1363.50, 1324.20, and 1233.79/cm confirms the presence of alkane, aldehyde, fluoride, nitro, and alkyl halide group, respectively. The remaining peak at 1022.65, 822.94, 764.05, and 662.95/cm indicated the presence of alcohol, aliphatic amines, aromatic compound, primary or secondary amines, and halogen group, respectively. In case of crude leaves powder, the peak observed at 3779.21, 3691.45, and 3282.84/cm represented the presence of phenol, amide, primary, or secondary amines as functional group, respectively. The peak at 2915.55, 2849.86, 2349.27, 1606.20, and 1443.36/cm showed the alkanes, carboxylic acid, alkenes, amino acid, and aromatic as functional group. The peak at 1364.60, 1314.63, 1233.56, and 1149.10/cm indicated the presence of alkanes, nitro compound, fluorides, aliphatic amines, alkyl halide, and ether as a functional group. The remaining peak at 1022.20, 828.21, 715.06, 659.77, and 594.32/cm confirms the presence of aliphatic amines, amines, primary or secondary amines, halogen, and alkyl halide as functional group. In case of crude berries powder, the peak observed at 3780.18, 3304.76, and 2918.89/cm indicated the presence of phenol, amine, amide, and alkanes as functional group, respectively. The peak at 2852.34, 1734.22, 1607.46, 1445.93, and 1325.32/cm shown the presence of alkanes, aldehyde, ketone, amino acid, aromatics, and nitro compound as functional group, respectively. The remaining peak at 1193.92, 1019.50, 762.70, 703.02, and 613.95/cm confirms the presence of functional group, namely, aliphatic amines, aromatic compound, aliphatic compound, and alkyl halide, respectively.

The absorption spectra of methanolic extract of *E. ribes* stem, leaves, and berries are shown in Fig. 2 and Table 2. The methanolic extract of *E. ribes* stem FTIR analysis confirms the presence of alcohol, amines, and amide at 3342.03/cm peak value. The peak at 2943.8 and 2831.95/cm indicated the presence of alkane. The remaining peak at 1613.16, 1455.63, 1120.44, 1020.16, and 627.716/cm represents the presence of amino acids, aromatic, ether compound, aliphatic amines, alcohols, alkynes, disulfides, and alkyl halide as a functional group, respectively. In case of methanolic extract of leaves, the peak observed at 3344.93 and 2936.09/cm shown the presence of alcohol, amines, amides, and alkanes, respectively. The peak at 2832.92/cm confirms the presence of alkanes and carboxylic acid. The remaining peak at 1644.98, 1455.99, 1021.12, 799.35, 635.43, and 624.323/cm indicates the presence of alkenes, aromatic, aliphatic amines, alcohols, aromatic compound, aldehyde, halogen, alkyl halide, alkynes, and disulfides as functional group, respectively. The methanolic extract of berries proved

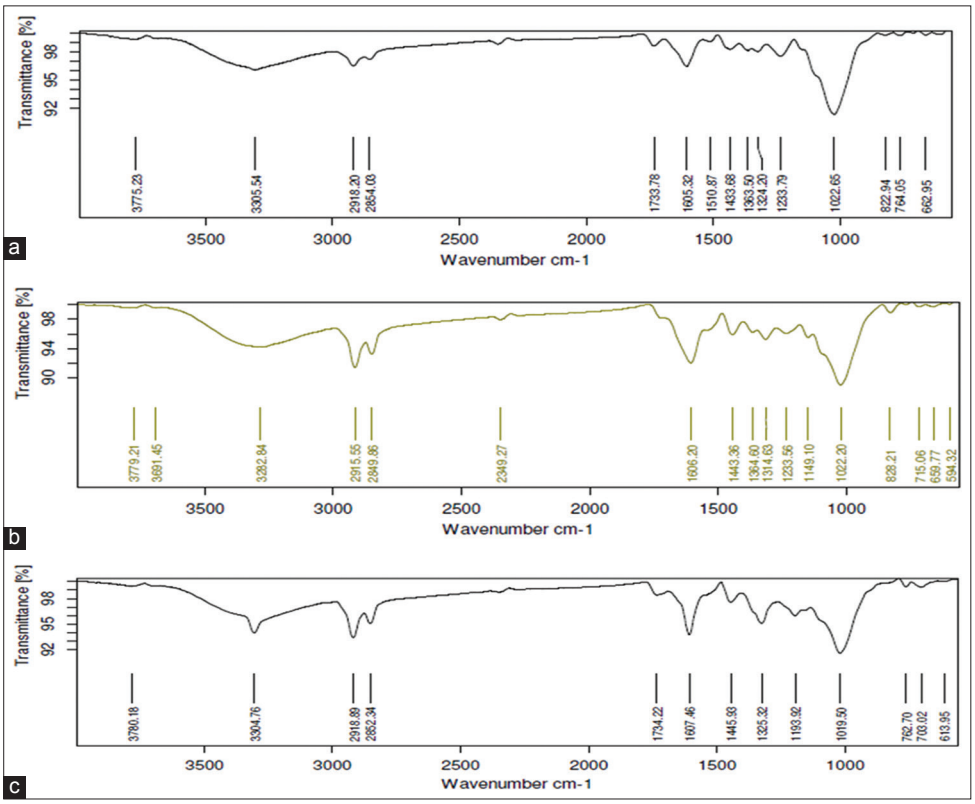

Fig. 1: Fourier transform Infrared spectrum analysis of *Embelia ribes* crude powder of (a) stem, (b) leaves, (c) berries

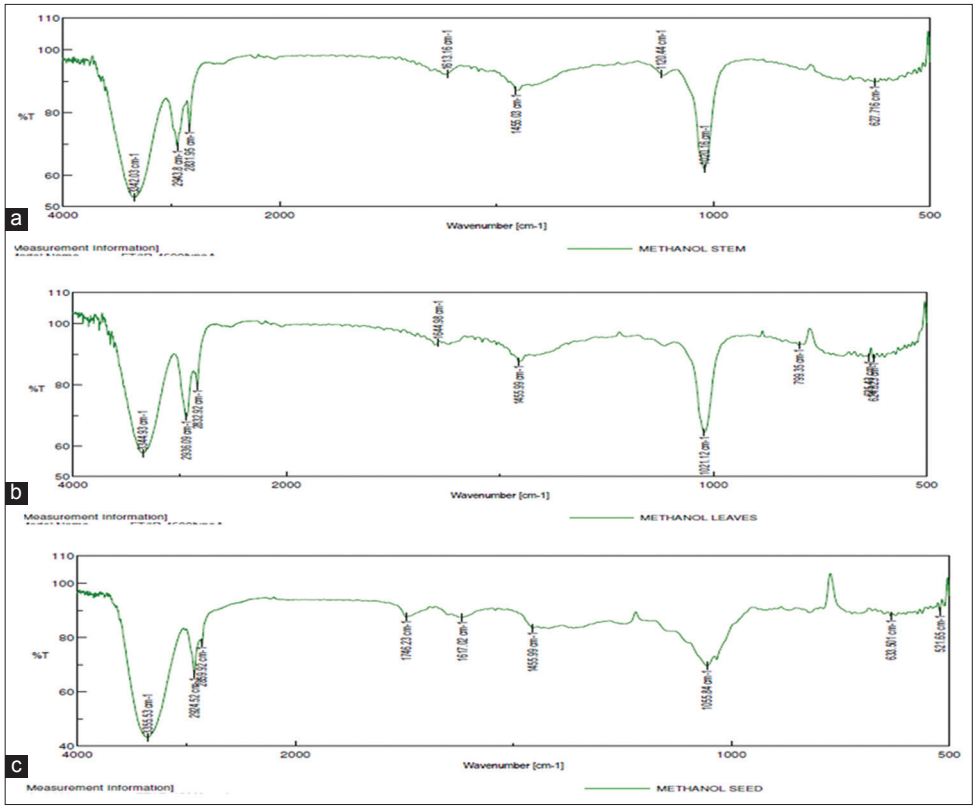

Fig. 2: Fourier transform Infrared spectrum analysis of *Embelia ribes* methanolic extract of (a) stem, (b) leaves, (c) berries

Table 1: FT-IR peak values and functional groups of crude powder of *Embelia ribes*

Serial number	Functional groups of the active component in crude powder of *Embelia ribes*					
	Stem peak value	Functional group	Leaves peak value	Functional group	Berries peak value	Functional group
1	3775.23	Phenol	3779.21	Phenol	3780.18	Phenol
2	3305.54	Amine, amide	3691.45	Amide	3304.76	Amine, amide
3	2918.20	Alkane	3282.84	Primary/secondary amines	2918.89	Alkane
4	2854.03	Alkane	2915.55	Alkane	2852.34	Alkane
5	1733.78	Aldehyde, ketones	2849.86	Carboxylic acid, alkane	1734.22	Aldehyde, ketone
6	1605.32	Amino acid	2349.27	Alkenes	1607.46	Amino acid
7	1510.87	Nitro compound, aromatic	1606.20	Amino acid	1445.93	Aromatic
8	1433.68	Aromatic	1443.36	Aromatic,	1325.32	Nitro
9	1363.50	Alkanes, aldehyde, fluoride	1364.60	Alkanes	1193.92	Aliphatic amines
10	1324.20	Nitro	1314.63	Alkane, nitro compound	1019.50	Aliphatic amines
11	1233.79	Fluoride, alkyl halide	1233.56	Fluorides, aliphatic amines, alkyl halide	762.70	Aromatic compound
12	1022.65	Alcohol, aliphatic amines	1149.10	Ether	703.02	Aliphatic compound
13	822.94	Alkyl halide, aliphatic amines	1022.20	Aliphatic amines	613.95	Alkyl halide
14	764.05	Aromatic compound, primary secondary amines	828.21	Amines		
15	662.95	Halogen	715.06	Primary/secondary amines		
16			659.77	Halogen		
17			594.32	Alkyl halide, halogen		

FTIR: Fourier transform infrared spectroscopy

the presence of alcohol, amines, amide, and alkane, which shown the major peak at 3355.53, 2924.52, and 2859.92/cm,respectively. The remaining peak at 1746.23, 1617.02, 1455.99, 1055.84, 633.501, and 521.65/cm indicates the presence of aldehyde, ester, amino acid, alkanes, aliphatic amines, alcohols, carboxylic acid, alkyl halide, and halogen as a functional group, respectively.

The absorption spectra of ethanolic extract of *E. ribes* stem, leaves, and berries are shown in Fig. 3 and Table 3. The results of *E. ribes* stem FTIR analysis confirmed the presence of amines, amide, alcohol, alkanes, and carboxylic acid at the peak value of 3354.57 and 2972.73/cm, respectively. The peak at 2884.12, 1379.82, and 1086.69/cm represents the presence of alkane, alkyl group, and aliphatic amines as functional group, respectively. The remaining peak at 1045.23, 879.381, 803.206, and 635.43/cm confirms the presence of aliphatic amines, primary or secondary amines, p-substituted alcohols or phenols, alkynes, alkyl halide, and disulfide as a functional group, respectively. In case of ethanolic extract of leaves, the peak observed at 3750.87, 3649.62, and 3387.35/cm confirms the presence of phenols, amide, and alcohol as a functional group,

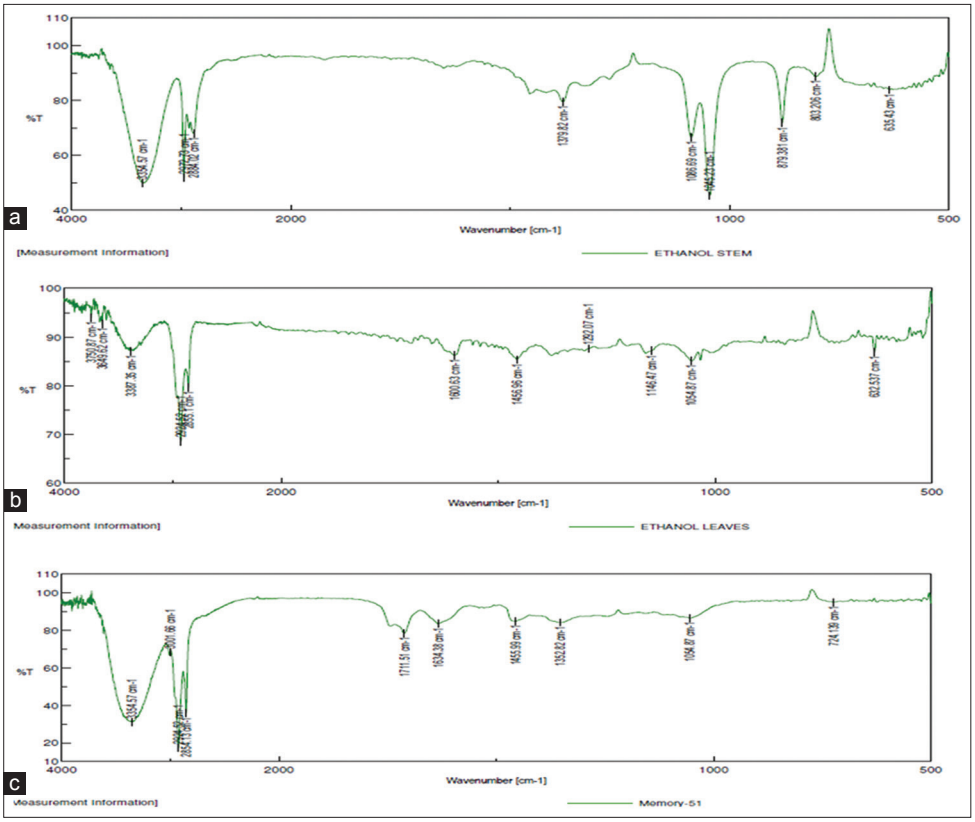

Fig. 3: Fourier transform Infrared spectrum analysis of *Embelia ribes* ethanolic extract of (a) stem, (b) leaves, (c) berries

Table 2: FTIR peak values and functional groups of methanolic extract of *Embelia ribes*

Serial number	Functional groups of the active component in methanolic extract *Embelia ribes*					
	Stem peak value	Functional group	Leaves peak value	Functional group	Berries peak value	Functional group
1	3342.03	Alcohol, amines, amides	3344.93	Alcohol, amines, amides	3355.53	Alcohol, amines, amides
2	2943.8	Alkane	2936.09	alkanes	2924.52	Alkanes
3	2831.95	Alkane	2832.92	Alkanes, carboxylic acids	2859.92	Alkanes
4	1613.16	Amino acids	1644.98	Alkenes	1746.23	Aldehyde, ester
5	1455.63	Aromatic	1455.99	Aromatic	1617.02	Amino acids
6	1120.44	Ether compound	1021.12	Aliphatic amines, alcohols	1455.99	Alkanes
7	1020.16	Aliphatic amines, alcohols	799.35	Aromatic compound, aldehyde, halogen	1055.84	Aliphatic amines, alcohols, carboxylic acids
8	627.716	Alkynes, disulfides, alkyl halide	635.43	Alkyl halide	633.501	Alkyl halide
9			624.323	Alkynes, disulfides, alkyl halide	521.65	Halogen, alkyl halide

FTIR: Fourier transform infrared spectroscopy

respectively. The peak at 2924.52 and 2855.1/cm represents the presence of alkanes and aldehyde as a functional group, respectively. The remaining peak at 1600.63, 1456.96, 1292.07, 1146.47, 1054.87, and 632.537 indicates the presence of amino acids, aromatics, alkanes, nitro compound, alkyl halide, carboxylic acid, aliphatic amines, alkynes, and disulfide as a functional group, respectively. The ethanolic extract of *E. ribes* berries shown the presence of amines, amide, alcohol, aldehyde, and alkane at the peak value of 3354.57, 2924.52, and 2854.13/cm, respectively. The peak at 1711.51, 1634.38, and 1455.99/cm indicates the presence of ketones, carboxylic acid, amino acid, aromatics, and alkanes as a functional group, respectively. The remaining peak at 1352.82, 1054.87, and 724.139/cm confirms the presence of nitro compound, carboxylic acid, aliphatic amines, and aliphatic compound, respectively. The absorption spectra of petroleum ether extract of *E. ribes* stem, leaves, and berries are shown in Fig. 4 and Table 4.

The petroleum ether extract of *E. ribes* stem FTIR analysis confirms the presence of phenols, amine, amide, alcohol, and alkanes at the peak of 3749.9, 3649.62, 3309.25, and 2955.38/cm, respectively. The peak at 2849.31, 1731.76, and 1614.13/cm shown the presence of alkanes, ketones, aldehyde, and amino acid as a functional group, respectively. The remaining peak at 1462.74, 1376.93, 1190.83, 1081.87, and 728.961/cm indicates the presence of alkanes, aromatics, nitro compounds, aliphatic amines, p-substituted alcohols, and phenols, respectively. In case of petroleum ether extract of leaves confirms the presence of phenols and alkane at the peak value of 3364.21, 2957.3, and 2923.56/cm, respectively. The peak at 2854.13 and 1705.73/cm represents the presence of alkanes, aldehyde, ketones, and esters as a functional group, respectively. The remaining peak at 1596.96, 1456.96, 1376.93, 1158.04, and 838.883/cm indicates the presence of primary amines, aromatics, alkanes, nitro compounds, alkyl halides, and primary or secondary amines as a functional group, respectively. The petroleum

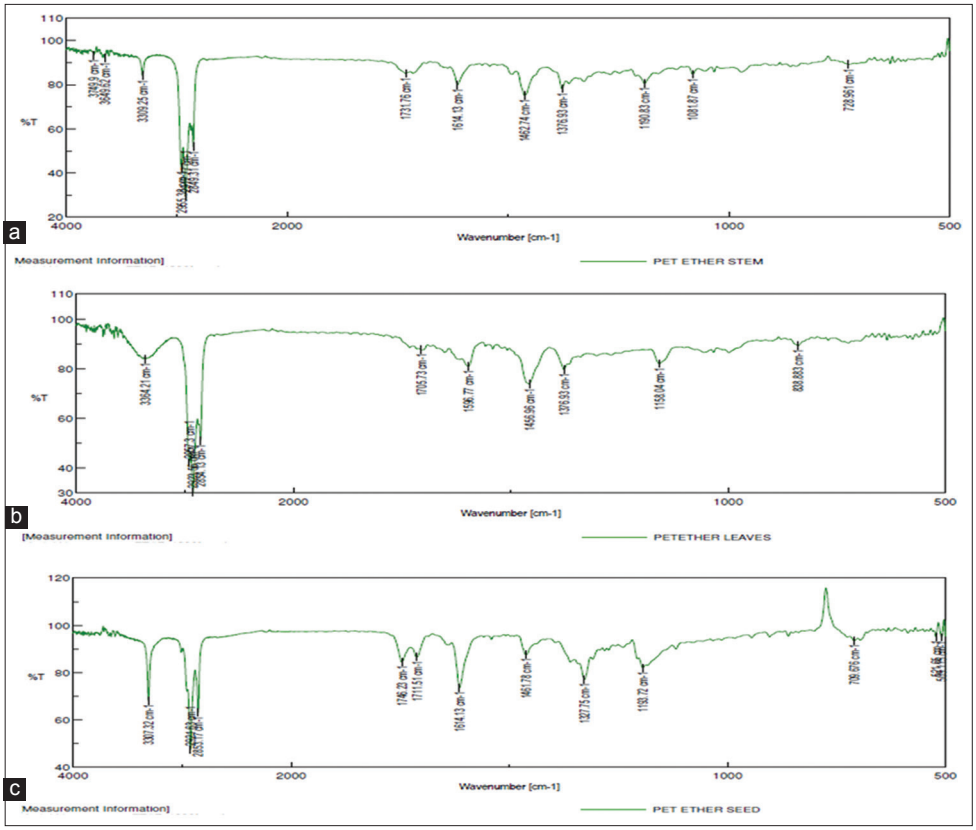

Fig. 4: Fourier transform infrared spectrum analysis of *Embelia ribes* petroleum ether extract of (a) stem, (b) leaves, (c) berries

Table 3: FTIR peak values and functional groups of ethanolic extract of *Embelia ribes*

Serial number	Functional groups of the active component in ethanolic extract *Embelia ribes*					
	Stem peak value	Functional group	Leaves peak value	Functional group	Berries peak value	Functional group
1	3354.57	Amines, amide, alcohol	3750.87	Phenols	3354.57	Amines, amide, alcohol
2	2972.73	Alkanes, carboxylic acid	3649.62	Amide	2924.52	Alkanes
3	2884.12	Alkane, alkyl group	3387.35	Alcohols, phenols	2854.13	Alkane, aldehyde
4	1379.82	Alkane	2924.52	Alkanes	1711.51	Ketone, carboxylic acid
5	1086.69	Aliphatic amines	2855.1	Alkane, aldehyde	1634.38	Amino acid
6	1045.23	Aliphatic amines	1600.63	Amino acid	1455.99	Aromatics, alkanes
7	879.381	Primary, secondary amines	1456.96	Aromatics, alkanes	1352.82	Nitro compound
8	803.206	p-substituted alcohols/phenols	1292.07	Nitro compound, alkyl halide	1054.87	Carboxylic acid, aliphatic amines
9	635.43	Alkynes, alkyl halide, disulphide	1146.47	Alkyl halides	724.139	Aliphatic compound
10			1054.87	Carboxylic acid, aliphatic amines		
11			632.537	Alkynes, alkyl halide, disulphide		

FTIR: Fourier transform infrared spectroscopy

ether extract of berries confirms the presence of alcohol, phenols, alkanes, and esters at the peak value of 3307.32, 2921.63, 2853.17, 1746.23, and 1711.51/cm, respectively. The remaining peak at 1614.13, 1461.78, 1327.75, 1193.72, 709.676, 521.65, and 509.115/cm indicates the presence of amino acid, alkanes, aromatics, nitro compounds, ether, aliphatic compound, alkyl halides, and aryl disulfides as a functional group, respectively.

Using FTIR spectrum, we can confirm the functional constituent's presence in the given parts and extract, identify the medicinal materials from the adulterate, and even evaluate the qualities of medicinal materials [17]. The results of the present FTIR spectroscopy revealed the functional constituents present in the crude powder, methanolic, ethanolic, and petroleum ether extracts of *E. ribes*. It also reveals the similarity and variation between the various parts of *E. ribes* based on the functional group presence and absorption spectrum.

From the spectra, we can see clearly that although they show substantial overlap of each absorption spectrum of various components, each band represents an overall overlap of some characteristic absorption peaks of functional groups in the samples [18]. The crude powder and dry ethanolic extracts of *Albizia lebbeck* leaves FTIR analysis results proved the presence of alcohols, phenols, alkanes, carboxylic acids, aromatics, ketones, and alkyl halides compounds which shows major

Table 4: FTIR peak values and functional groups of petroleum ether extract of *Embelia ribes*

Serial number	Functional groups of the active component in petroleum ether extract *Embelia ribes*					
	Stem peak value	Functional group	Leaves peak value	Functional group	Berries peak value	Functional group
1	3749.9	Phenols	3364.21	Phenols	3307.32	Alcohol, phenols
2	3649.62	Amine, amide	2957.3	Alkane	2921.63	Alkanes
3	3309.25	Alcohols, phenols, amines, amide	2923.56	Alkane	2853.17	Alkanes
4	2955.38	Alkanes	2854.13	Alkanes	1746.23	Esters
5	2849.31	Alkanes	1705.73	Aldehyde, ketone, ester	1711.51	Esters
6	1731.76	Ketones, aldehyde	1596.77	Primary amines, aromatics	1614.13	Amino acid
7	1614.13	Amino acid	1456.96	Alkanes, aromatics	1461.78	Alkanes, aromatics
8	1462.74	Alkanes, aromatics	1376.93	Nitro compounds	1327.75	Nitro compounds
9	1376.93	Alkane, Nitro compounds	1158.04	Alkyl halides	1193.72	Ether
10	1190.83	Aliphatic amines	838.883	Primary, secondary amines	709.676	Aliphatic compound
11	1081.87	Aliphatic amines			521.65	Alkyl halides
12	728.961	p-substituted alcohols, phenols			509.115	Aryl disulfides

FTIR: Fourier transform infrared spectroscopy

peaks at 3370.19, 2955.65, 2925.68, 2853.40, 1739.72, 1463.02, and 506.57/cm, respectively [20].

FTIR spectral analysis of plant parts such as flowers, leaves, stem, and roots of *Aerva lanata* showed the presence of characteristic functional groups of alcohols, phenols, alkanes, carboxylic acids, aldehydes, alkenes, nitro compounds, alcohols, carboxylic acids, esters, ethers, aliphatic amines, and alkyl halides compounds derivatives which were responsible for medicinal properties of the plant [19]. Similar type of analysis has been documented in *Ichnocarpus frutescens* [21]. The methanol extract of *Limonia acidissima* fruit contained alcohols, phenols, alkanes, amino acids, α, β-unsaturated esters, alkenes, nitro compounds, aromatics, aliphatic amines, carboxylic acid, alkenes, and alkyl halides compounds [22], and the ethanol extracts of *Ipomoea obscura* (L.) showed the presence of most of the secondary metabolites in the plant leaves [23]. Similarly, *Cayratia trifolia* plant stems ethanolic extract holds more phytochemical and bioactive compounds which were confirmed using FTIR [24]. The methanolic extract of fruit pulp of *Feronia limonia* shown the presence of phenolic, aromatic, and aliphatic functional group [25].

CONCLUSION

The results of the present study showed the presence of amino acids, amide, alkanes, alkenes, alkynes, aldehyde, ketones, fluorides, carboxylic acids, alcohols, esters, ethers, aromatics, aliphatic amines, p-substituted alcohols or phenols, disulfide, halogens, alkyl halides, and nitro compounds as functional group. It indicates the medicinal property of *E. ribes*, which can be utilized for various pharmaceutical purposes. Further advanced spectroscopic studies are needed to elucidate the structure and identification of active principles present in the *E. ribes*.

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