A COMPARATIVE FOURIER TRANSFORM INFRARED SPECTROSCOPIC ASSESSMENT OF LEAF EXTRACT IN AVERRHOA BILIMBI L. AND AVERRHOA CARAMBOLA L.

Hari N
Asst. Professor, Department of Botany, C M S College Kottayam, Kottayam, Kerala, India
Email: drhari@cmscollege.ac.in
C Priya
Ph.D. Research Scholar, Department of Botany, C M S College Kottayam, Kerala, India
V Kavya
Student, C M S College Kottayam, Kottayam, Kerala, India
E Besteena
Student, C M S College Kottayam, Kottayam, Kerala, India
Email: besteena2014@gmail.com

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Abstract: The study helps to assess and compare the probable functional groups present in leaf extract by using various solvent extracts in *Averrhoa bilimbi* and *Averrhoa carambola*. *Averrhoa bilimbi*, popularly known as bilimbi, cucumber tree, tree sorrel is a fruit-bearing attractive, long-lived evergreen tree. *Averrhoa carambola* is an attractive, small, slow-growing evergreen tree and is commonly known as Star fruit because of unique, star-like shaped fruit. They are widely distributed in tropical and subtropical regions of the world. The plant parts were used in traditional medicine for the treatment of a variety of ailments. The IR spectrum in mid-infrared regions was used to compare the various functional groups present in the species belonging to the family Oxalidaceae. The Family Oxalidaceae is one of the very important medicinally significant biological sources but ignored and less studied. The presence of C-N, C=S, C-O, S=O, P-OR, Si-OR, NH_2, C=C, C-H, O-H, N-H, S-OR, C-F, C=O, N-O, C-Cl, C-N, and P-H were identified. The bonding structures represented for the presence of alkanes, alkenes, alkyl halides, alcohols, aldehydes, amines, amides, aromatic, anhydrides, carboxylic acids, carbonyl, ethers, esters, ketones, silane, sulfonyl chloride, sulfoxide, a nitro compound in both the species. The different groups seen in both the species are thiocarbonyl, phenol, sulfone in *A. bilimbi* and phosphine in *A. carambola*. The method was performed on an FTIR Spectroscopy with the scan range from 600 to 4000 cm^{-1}. The results ascertain that in *Averrhoa bilimbi* and *Averrhoa carambola* are having similar functional groups in the leaves with their phytoconstituents and subjecting it to biological activity.

Keywords: *Averrhoa Bilimbi* L., *Averrhoa Carambola* L., Leaf, FTIR, Spectroscopy, Functional Groups.

Introduction: Nature has been a source of medicinal compounds for thousands of years. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made a significant contribution towards human health. Almost every species of medicinal plants contains more than one active compound and it is necessary to know the composition before other studies are being undertaken. Family Oxalidaceae is one of the very important medicinally significant biological sources but ignored and less studied[(12)]. Oxalidaceae, sometimes called the 'wood sorrel' family comprises eight genera and about 900 species. They are widely distributed in tropical and subtropical regions of the world. *Averrhoa bilimbi* L. and *Averrhoa carambola* L. are the two species of the genus *Averrhoa* L. *A. bilimbi* popularly known as bilimbi, cucumber tree, tree sorrel is a fruit-bearing attractive, long-lived evergreen tree whereas *A. carambola* is an attractive, small, slow-growing evergreen tree and is commonly known as Star fruit because of unique, star-like shaped fruit. The species name '*bilimbi*'

derives from the Malay vernacular name for the species and the word 'carambola' is derived from the Sanskrit karmaranga, meaning "food appetizer"[8]. They differ from each other in appearance, manner of fruiting, size, flavor, and uses. The plant parts were used in traditional medicine for the treatment of a variety of ailments. *A.bilimbi* leaves extract acts as an effective antibacterial agent and have good inhibitory activity against certain pathogens. *A.carambola* has shown more pharmacological activities like anti- helminth, antimalarial and antipyretic properties[10].

FTIR (Fourier Transform Infrared Spectrophotometer) is one of the widely used methods to identify the chemical constituents and elucidate the compound structures to propose in medicinal purposes. At present, particularly in phytochemistry, FTIR has been exercised to identify the concrete structure of certain plant secondary metabolites.The preliminary phytochemical screening of the leaves extracts of *A. bilimbi* revealed the presence of alkaloids, tannins, saponins, flavonoids, cardiac glycosides, glycosides, triterpenes, phenols, and carbohydrates whereas *A. carambola* revealed the presence of saponins, alkaloids, flavonoids, and tannins[8]. A survey of literature revealed that the FTIR analysis of functional groups was not done so far with the medicinal plants such as *A. bilimbi* and *A. carambola*. Hence, an attempt is made in the present study to assess and compare the probable functional groups present in dry leaf powder and leaf extract by using various solvent extracts (Petroleum Ether, Chloroform, Acetone, Ethanol) of the *A. bilimbi* and *A. carambola*.

2. Materials and Methods:

Materials: *Collection of Plant Materials:* The plants selected were *Averrhoa bilimbi* L. and *Averrhoa carambola* L. The plant materials were collected from C M S College Kottayam, Kottayam district, Kerala (Fig. 1). This was authenticated for its species and the corresponding family.

Kingdom	***Plantae***
Subkingdom	*Tracheobionta*
Superdivision	*Spermatophyta*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Subclass	*Rosidae*
Order	*Geraniales*
Family	*Oxalidaceae*
Genus	*Averrhoa*
Species	A*verrhoabilimbi* *Averrhoa carambola*

Characters	***Averrhoabilimbi***	***Averrhoa carambola***
Habit	Evergreen tree	Evergreen tree
Origin	Southeast Asia	Ceylon and Moluccas
Habitat	Lowland primary and secondary forests	Humid forests and woodland on sandy loam.
Climate	Humid tropical lowlands	Hot humid tropics
Importance	Food, medicines, commercial purposes	Medicines, cultivation of fruits in gardens, Ornamental purposes

Description: A.bilimbi is a small tree that grows up to 15 m high with sparsely arranged branches. It has compound leaves with twenty–forty leaflets each and 5–10 cm long. The leaves are hairy with pinnate shapes and form clusters at the end of branches. The tree is cauliflorous with 18–68 flowers in panicles that form on the trunk and other branches. The flowers are with petal 10–30 m long, yellowish-green to reddish-purple. The fruits are produced on the bare stem and trunk.The fruits are greenish with a firm and juicy flesh which becomes soft on ripening. The fruit juice is sour and extremely acidic[2].

A.carambola is a small tree growing to a height of 6 meters or less. Leaves are pinnate, about 15 centimeters long. Leaflets are smooth, usually in 5 pairs. Pannicles are small, axillary, and bell-shaped, 5 to 6 millimeters long. Flowers are red and white appear on bare branches or at leaf bases. The calyx is reddish-purple. Petals are purple to bright purple, often margined with white. The fruit is fleshy, green to greenish-yellow, about 6 centimeters long, with 5 longitudinals, sharp and angular lobes. Seeds are arillate. Seedlings have been known to bear in 3 years[8].

Fig. 1.A: Matured Plant, Flower, Fruit of A*verrhoa Bilimbi*

Fig. 1.B: Matured Plant, Flower, Fruit of A*verrhoa Carambola*

2.2. Methods:

Preparation of Plant Extracts: The mature leaves were collected from the mother plant; Leaves were detached and dried in shade at ambient temperature for three weeks. The well-dried samples were powdered separately by using an electric blender. The powdered plant part (leaves) 1gm each was extracted in 10 ml of petroleum ether, chloroform, acetone, ethanol with continuous shaking on a mechanical shaker for 24 hrs at room temperature. The extracts were then filtered through Whatsman No.1 filter paper. The extracts were used for further analysis.

Preparation of Sample for Infra-Red Spectrophotometer (FTIR) Analysis: The FTIR spectrum was used to identify the functional groups of the active components in the plant sample based on the peak value in the region of Infrared radiation. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. The extracts were encapsulated separately in KBr pellet, to prepare translucent sample discs. Each sample was loaded in FTIR Spectroscopy with a scan range from 600 to 4000 cm^{-1} (Shimadzu, Model No. IR- Prestige 21).

3. Results: The leaf extracts of *Averrhoa bilimbi* and *Averrhoa carambola* as a dry powder and prepared in petroleum ether,chloroform, acetone, ethanol gave the following absorption peaks(Figure 2 to 6)&Tables 1 to 10)

FTIR Spectral Data Interpretation:

FTIR analysis in Dry Leaf Powder: The absorption spectra of *Averrhoa bilimbi* in dried leaf powder (Figure-2 & Table- 1) exhibited peaks at 1039.63,1627.92, 2922.15, 3327.20. The absorption spectra of *Averrhoa carambola* (Figure-2& Table-2) exhibited peaks at 1037.70, 1645, 2922.15, 3342.63.The peaks of *Averrhoa bilimbi* represented the presence of amines (C-N, NH_2stretch), thiocarbonyl (C=S stretch), sulfoxide (S=O stretch), esters (P-OR stretch) alkene (C=C stretch), alkane (C-H stretch), the carboxylic acid (O-H stretch), alcohol (O-H stretch), amide (N-H stretch), phenols (O-H stretch) and silane (Si-OR stretch). The Peak of *Averrhoa carambola* represented the presence of ester (C-O stretch),sulfoxide (S=O stretch) and silane(Si-OR stretch),amines (NH_2, N-H stretch), alkene (C=C stretch) and amides(C=O stretch), alkane (C-H stretch) and carboxylic acid (O-H stretch) and alcohol (O-H stretch).

The infrared spectrum of *Averrhoa bilimbi* and *Averrhoa carambola* with the given frequency ranges from 1000 –3600; the peaks are probably of alkanes, alkenes, alcohol, amines, amide, thiocarbonyl, sulfoxide, esters, carboxylic acid, and silane The stretches such as C-N, C=S, S=O, P-OR, Si-OR,NH_2, C=C, C-H, O- H, N-H, C-O with the nearest range representing the same functional groups reported by N.hari etal[9&10]. and IOCD[6].

FTIR Analysis in Petroleum Ether: The absorption spectra of *Averrhoa bilimbi* in petroleum ether (Figure-3 & Table- 3) exhibited peaks at 769.99, 1064.70, 1460.11, 1716.64, 2918.29. The absorption spectra of *Averrhoa carambola*(Figure-3& Table-4) exhibited peaks at 769.59, 1068.56, 1462.04, 1728.21, 2960.73. The peaks of *Averrhoa bilimbi* represented the presence of amines (NH_2 and N-H stretch), esters (S-OR stretch), thiocarbonyl (C=S stretch), alcohol (C-O stretch), alkyl halide (C-F stretch) and ether (C-O stretch), alkane (C-H bending) and aromatic (C=C stretch), aldehydes and ketones(C=O stretch) and carboxylic acid (C=O stretch). The Peaks of *Averrhoa carambola* represented the presence of alkyl halide (C-Cl stretch),esters (S-OR,C-O stretch), alcohol (C-O stretch), anhydrides(O-C stretch) and amines (C-N stretch),aromatic(C=C stretch) and alkane (C-H bending), carbonyl(C=O stretch), aldehyde(C=O stretch), and carboxylic acid (O-H stretch).

The infrared spectrum of *Averrhoa bilimbi* and *Averrhoa carambola* with a frequency ranges from 660 – 3000; the peaks are probably of alkane, amines, alkyl halide,anhydrides, esters, thiocarbonyl, alcohol, aldehydes, ketones, and carboxylic acids. The stretches such as NH2, N-H, S-OR, C=S,C-O, C-F, C-O, C=C, C=O, C-H,C-O, and O-H bend with the nearest range representing the same functional groups reported by Adina et al., (2012)(1), N.hari etal.,[9&10] and IOCD[6].

FTIR analysis in Chloroform Extract: The absorption spectra of *Averrhoa bilimbi* L., (Figure-4 & Table-5) exhibited peaks at 769.99, 1217.08, 1714.71, 2924.08. The absorption spectra of *Averrhoa carambola* L., (Figure-4& Table-6) exhibited peaks at 769.99, 1217.08, 1716.71, 2922.15. The peaks of *Averrhoa bilimbi* represented the presence of esters (S-OR stretch), amines (NH_2 and N-H) and alkyl halides (C-H,C-F stretch),ether (C-O stretch), the carboxylic acid (C-O stretch), carbonyl (C=O stretch), ketone (acyclic stretch), alkane (C-H stretch).The Peaks of *Averrhoa carambola* represented the presence of alkene(C=H bending),alkyl halides(C-Cl, C-F stretch), esters(S-OR stretch), amines (C-N stretch), the carboxylic acid (C-O,C=O,O-H stretch), ether (C-O stretch), carbonyl (C=O stretch), ketone (acyclic stretch), alkane (C-H stretch).

The infrared spectrum with a frequency ranges from 650 –3300; the peaks are probably of alkane, amines, esters, alkyl halides, ether, carboxylic acid, carbonyl, and ketone. The stretches such as S-OR, NH2, N-H, C-Cl, C-F, C-N, C-O, C=O, O-H, and C-H with the nearest range representing the same functional groups reported by N.hari etal.,[9&10]IOCD[6] and Robert et.al.(2016)[11].

FTIR Analysis in Acetone Extract: The absorption spectra of *Averrhoa bilimbi* L., (Figure-5& Table- 7) exhibited peaks at 1224.79, 1361.74, 1708.93, 2922.15. The absorption spectra of *Averrhoa carambola* L., (Figure-5 & Table-8) exhibited peaks at 1222.86, 1361.74, 1708.93, 2920.22, 3417.86. The peaks of *Averrhoa bilimbi* represented the presence of alkyl halide (C-F stretch), amine (C-N stretch), ether (C-O stretch), the carboxylic acid (C-O stretch), anhydride (C-O stretch), alcohols(O-H), phenols (C-O stretch),

sulfonyl chloride(S=O stretch), nitro (N-O stretch), alkane (C-H bend), carbonyl (C=O stretch), ketone (acyclic stretch). The Peaks of *Averrhoa carambola* represented the presence of carboxylic acid (C-O C=O,O-H stretch),ester(C-O stretch),amines (C-N, NH_2 and N-Hstretch), phosphine(P-H stretch), sulfonyl chloride (S=O stretch), aldehyde(C=O stretch), ketone (C=O stretch), alkane (C-H stretch), alcohol(O-H stretch), amide(N-H stretch).

The infrared spectrum with a frequency ranges from 970 –3500; the peaks are probably of alkane, amine, anhydride, alcohols and phenols, alkyl halide, ether, nitro, carbonyl, carboxylic acid, ketone, and sulfonyl chloride. The stretches such as C-F, C-N, C-O, S=O, N-O, C=O, N-H, NH_2,P-H, and O-H with the nearest range representing the same functional groups reported by N.hari etal.[9&10], IOCD[6], Adina *et.al.*(2012)[1] and Robert *et.al.*(2016) [12].

FTIR analysis in Ethanol Extract: The absorption spectra of *Averrhoa bilimbi* L., (Figure-6 &Table-9) exhibited peaks at 1045.41, 1384.88, 1662.63, 2974.23, 3361.92. The absorption spectra of *Averrhoa carambola* L., (Figure-6 & Table-10) exhibited peaks at 1045.41, 1384.88, 1660.71, 2974.23, 3358.06. The peaks of *Averrhoa bilimbi* represented the presence of amines (C-N stretch), sulfoxide (S=O stretch), esters (P-OR stretch), anhydrides (O-C stretch), alkane (C-H bending), amine (C-N stretch), aromatic (C=C stretch), sulfone (S=O stretch) and sulfate (S=O stretch), alkenes (C=C stretch) and amides (C=O stretch), the carboxylic acid (O-H stretch), alcohol(O-H stretch), phenols (O-H stretch). The Peaks of *Averrhoa carambola* represented the presence of alkane (C-H stretch), alkyl halide (C-F stretch) and ether (C-O stretch), nitro (N-O stretch), amides (C=O,N-H stretch), the carboxylic acid (O-H stretch), alcohol (O-H stretch) and amine (N-H stretch).

The infrared spectrum with a frequency ranges from 1000 –3550; the peaks are probably of alkanes, amides, amines, anhydrides, esters, sulfone, sulfate, sulfoxide, alcohols, and phenols. The C-H bend and stretches such as C-N, S=O, C-F, C-O, P-OR, C=C, C-H, N-H, and O-H with the nearest range representing the same functional groups reported by N.hari etal.,[9&10],IOCD[6] and Adina *et.al.*(2012)[1] .

Table 1: FTIR Peak Values and Functional Groups in the Dry Powder of *Averrhoa bilimbi* L., Leaves

S. No.	Wave number(cm^{-1})	Frequency ranges(cm^{-1})	Functional group
1	1039.63	1000– 1250	Amines,Thiocarbonyl, Sulfoxide, Esters, Silane
2	1627.92	1550 – 1650	Amines and Alkene
3	2922.15	2850 – 3000	Alkane and Carboxylic acid
4	3327.20	3300 – 3600	Alcohol, Amide, Amines, Phenols

Table 2: FTIR Peak Values and Functional Groups in the Dry Powder of *Averrhoa Carambola* L., Leaves

S. No.	Wave number(cm^{-1})	Frequency ranges(cm^{-1})	Functional group
1	1037.70	1000– 1300	Esters, Sulfoxide,Silane
2	1645	1630-1670	Amines,Alkene,Amide
3	2922.15	2922.15	Alkane and Carboxylic acid
4	3342.63	3200 – 3400	Amines,Alcohol, Amide

Table 3: FTIR Peak Values and Functional Groups in Petroleum Ether Extract of *Averrhoa Bilimbi* L., Leaves

S. No.	Wave number(cm^{-1})	Frequency ranges(cm^{-1})	Functional group
1	769.99	660 – 900	Amines and Esters
2	1064.70	1050 - 1200	Thiocarbonyl, Alcohol, Alkyl halide, and Ether
3	1460.11	1350 - 1470	Alkane and Aromatic
4	1716.64	1710 - 1720	Aldehydes, Ketones, and Carboxylic acid
5	2918.29	2850 - 3000	Alkane and Carboxylic Acid

Table 4: FTIR Peak Values and Functional Groups in Petroleum Ether Extract of *Averrhoa Carambola* L., Leaves

S. No.	Wave number(cm^{-1})	Frequency ranges(cm^{-1})	Functional group
1	769.59	660 – 900	Amines and Esters
2	1068.56	1000 – 1300	Alcohol,Ester,Anhydrides,Amines
3	1462.04	1475-1600	Aromatic and alkane
4	1728.21	1720-1740	Carbonyl,Aldehydes
5	2960.73	2850 – 3000	Alkane and Carboxylic Acid

Table 5: FTIR Peak Values and Functional Groups in Chloroform Extract of *Averrhoa Bilimbi* L., leaves

S. No.	Wave number(cm-1)	Frequency ranges(cm-1)	Functional group
1	769.99	700 – 900	Esters, Amines, Alkyl Halides
2	1217.08	1020 - 1250	Alkyl halide, Amines, Ether, Carboxylic acid, Ester
3	1714.71	1710 - 1720	Carbonyl, Carboxylic acid, Ketone
4	2924.08	2500 – 3300	Carboxylic acid, Alkane

Table 6: FTIR Peak Values and Functional Groups in Chloroform Extract of *Averrhoa Carambola* L., Leaves

S. No.	Wave number(cm^{-1})	Frequency ranges(cm^{-1})	Functional group
1	769.99	650-1000	Alkene,Alkyl Halides, Esters,
2	1217.08	1000-1300	Amines, Carboxylic acid, Alkyl halide,Ether
3	1716.64	1700-1725	Carbonyl, Carboxylic acid, Ketone
4	2922.15	2850 – 3000	Carboxylic acid, Alkane

Table 7: FTIR Peak Values and Functional Groups in Acetone Extract of *Averrhoa Bilimbi* L., Leaves

S. No	Wave number (cm^{-1})	Frequency ranges(cm^{-1})	Functional group
1	1224.79	970 - 1250	Alkyl Halide, Amine, Ether, Carboxylic acid, Anhydride, Alcohol, and Phenols
2	1361.74	1365±5	Sulfonyl chloride, Nitro, Alkyl halide, and Alkane
3	1708.93	1700 - 1725	Carbonyl, Carboxylic acid and Ketone
4	2922.15	2850 – 3000	Alkane and Carboxylic acid

Table 8: FTIR Peak Values and Functional Groups in Acetone Extract of *Averrhoa Carambola* L., Leaves

S. No.	Wave number(cm^{-1})	Frequency ranges(cm^{-1})	Functional group
1	1224.86	1000-1300	Carboxylic acid,Ester,Amines,Phosphine
2	1361.74	1300-1375	Amines,Sulfonyl chloride
3	1708.93	1705-1725	Aldehyde, Ketone Carboxylic acid
4	2920.15	2850 – 3000	Carboxylic acid and Alkene
5	3417.86	3100-3500	Amines,Alcohol,Amide

Table 9: FTIR Peak Values and Functional Groups in Ethanol Extract of *Averrhoa Bilimbi* L.,Leaves

S. No.	Wave number(cm^{-1})	Frequency ranges(cm^{-1})	Functional group
1	1045.41	1000 – 1250	Amines, Sulfoxide, Esters,Anhydrides
2	1384.44	1350 - 1470	Alkane, Amine, Aromatic, Sulfone, Sulfate
3	1662.63	1630 - 1670	Alkane and Amides
4	2974.23	2850 - 3000	Alkane and Carboxylic acid
5	3361.92	3200 - 3550	Alcohols, Phenols and Amine

Table 10: FTIR Peak Values and Functional Groups in Ethanol Extract of *Averrhoa Carambola* L., Leaves

S. No.	Wave number(cm^{-1})	Frequency ranges(cm^{-1})	Functional group
1	1045.41	1000 – 1300	Alkane, Alkyl Halide, And Ether
2	1384.88	1350 - 1470	Alkane ,Alkyl Halide And Nitro
3	1660.71	1630 - 1680	Alkenes and Amides
4	2974.23	2850 - 3000	Alkane and Carboxylic acid
5	3358.06	3200-3400	Alcohols, Amine and Amide

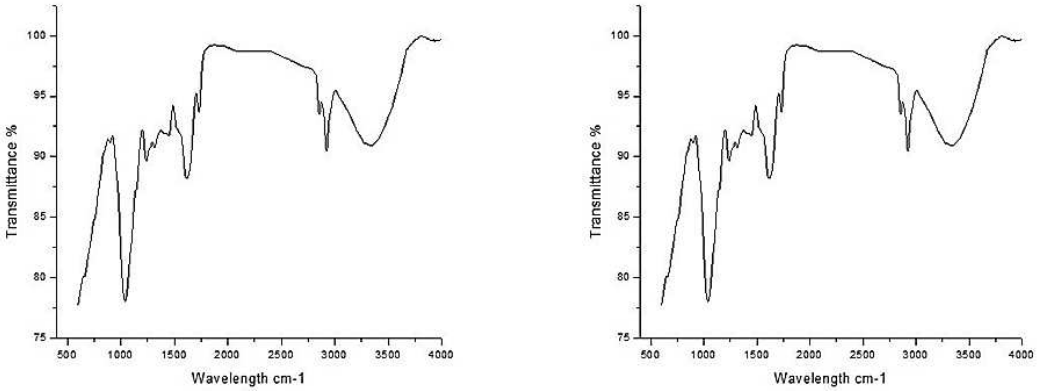

Figure 2: FTIR Analysis of *Averrhoa Bilimbi* and *Averrhoa Carambola* Leaves as a Dry Powder

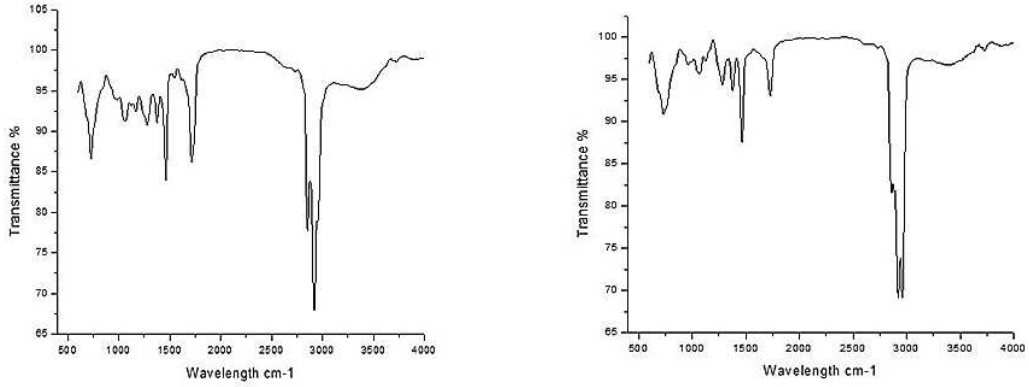

Figure 3: FTIR Analysis of *Averrhoa Bilimbi* and *Averrhoa Carambola* Leaves in Petroleum Ether Extract

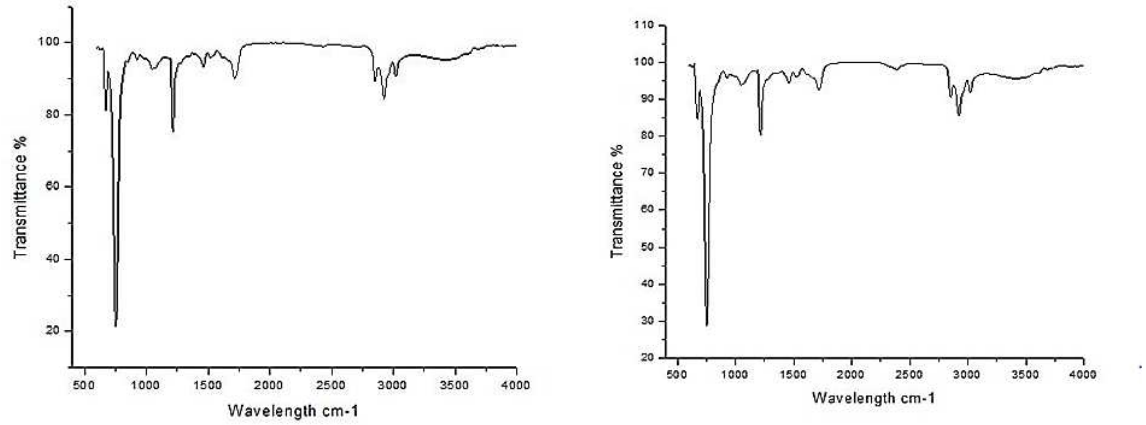

Figure 4: FTIR Analysis of *Averrhoa Bilimbi* and *Averrhoa Carambola* Leaves in Chloroform Extract

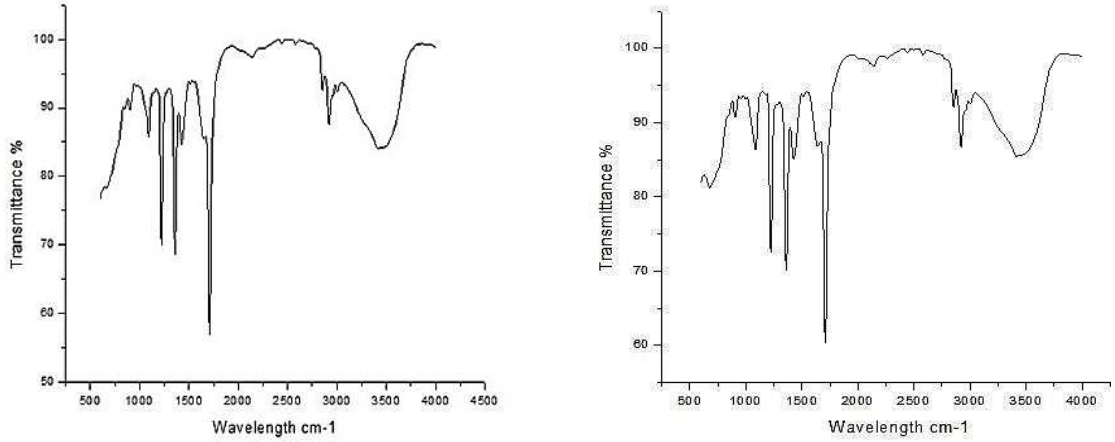

Figure 5: FTIR Analysis of *Averrhoa Bilimbi* and *Averrhoa Carambola* Leaves in Acetone Extract

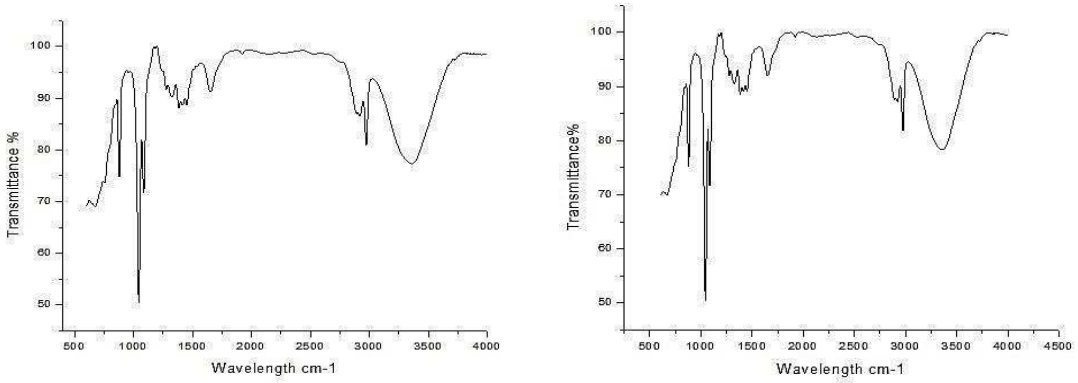

Figure 6: FTIR Analysis of *Averrhoa Bilimbi* and *Averrhoa Carambola* Leaves in Ethanol Extract

4. Conclusion: The various functional groups observed in the different extracts probably indicate the presence of alkanes, alkenes, alkyl halides, alcohols, aldehyde, amines, amides, aromatic, anhydrides, carboxylic acids, carbonyl, ethers, esters, ketones, silane, sulfonyl chloride, sulfoxide, a nitro compound in both the species. The different groups seen in both the species are thiocarbonyl, phenol, sulfone in *A.bilimbi* and phosphine in *A. carambola* with their phytoconstituents and subjecting it to the biological

activity will give fruitful results. By using the FT-IR spectrum, we can confirm the functional constituent's presence in the given part and extract, identify the medicinal materials from the adulterate, and even evaluate the qualities of medicinal materials. Many researchers applied the FTIR spectrum as a tool for distinguishing closely associated plants. So it is recommended as a stepping tool for further studies for the identification, isolation of compounds and their biological application.

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