Phytochemical Screening by FTIR Spectroscopic Analysis of Some Indian Medicinal Plants

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Abstract:

The present study is aimed to analyse the ethanol and aqueous extracts of leaves of Azardirachta indica, Vitex nigundo and seeds of Trigonella foenum graecum through FTIR spectroscopy method. The FTIR spectroscopic studies revealed different characteristic peak values with different functional groups in the extracts. The FTIR analysis of leaf extracts of Azardirachta indica, Vitex nigundo and seeds of Trigonella foenum graecum extract confirmed the presence of amide, alcohols, phenols, alkanes, carboxylic acids, aldehydes, ketones, alkenes, primary amines, aromatics, esters, ethers, alkyl halides and aliphatic amines compounds, which shows major peaks. The FTIR method was performed on a spectrophotometer system, which was used to detect the characteristic peak values and their functional groups. The results of the present study generated the FTIR spectrum profile for these medicinally important plants and these can be used in the industry. The nature of therapeutic action of theseplants as understood from its chemical properties could be a novel approach in developing cost effective and safe herbal formulations to deal with dermal disorders.

Keywords: *Azardirachta indica; Vitex nigundo; Trigonella foenum graecum ; FTIR Spectroscopy; Functional groups.*

Introduction:

Bacteria are unable to develop resistance to multiple chemically complex chemically complex phytochemical present in plant extracts. The increasing failure of chemotherapeutics with antibiotic resistance by pathogenic bacteria has led to the different medicinal plants for their antimicrobial activity. Traditional medicines play a very important role around the globe in health services. Plants are considered to be a vital source of potentially useful phytoconstituents for the developments of new drugs. Many plants are containing natural antibacterial compounds which are used to treat most of the bacterial infections as natural medicine [1]. India is one of top twelve megadiversity centers and the wealth of medicinal and aromatic plants. The efficacy depends on the use of proper plant part and its biological activities which in turn depends up on the presence of required amount of different phytoconstituents. In Indian system of medicine *Azardirachta indica, vitex nigundo, trigonella foenum graecum* has been mentioned as a remedy for the treatment of different skin diseases and ailments. Based on this folkloric use, the present study was designed to analyze the different functional groups present in these plant extracts. . Identification of the chemical nature of constituents present in the medicinal plants will provide valuable information related to functional groups responsible for its therapeutic actions. [2]Natural product's properties are related to certain classes of compounds such as alkaloids, flavonoids, essential oils and others. During the decades near-infrared spectroscopy has become one of the most important

tools for the analysis due to following reasons: it represents a non-invasive analytical tool allowing a fast and simultaneous qualitative as well as quantitative determination of natural products and their constituents. The infrared spectrum is formed as a consequence of the absorption of electromagnetic radiation at frequencies that correlate to the vibration of specific sets of chemical bonds from within a molecule. [3]The present study is therefore aimed at identifying the functional groups and phytoconstituents present in ethanol and aqueous extracts of different plants by FTIR spectroscopic analysis that could logically enable its applied aspect in present day's herbal medicines.(3,4)

Materials and methods:

Leaves of *Azardirachta indica Linn* and *vitex nigundo* Linn are collected from local region of Latur in the month of august to November and seeds of *Trigonella foenum graecum* were collected from local market of Latur. The plant parts were identified by Botanist at department of botany, Dayanand Science College, Latur. Voucher specimens were deposited at Dayanand science college, Latur. Plant materials were washed thoroughly with distilled water to remove any earthy matter or remove the dirt and any other contaminations. The plant material was dried at room temperature under shade to retain their fresh green colour and also to prevent the loss of active compounds. The dried plant material was grinded to coarse powder and stored in air tight container for further use.

Extraction:

The air dried powdered leaves (30g) of *Vitex nigundo linn, Azardirachta indica linn and* seeds of *trigonella foenum graecum* Extracted with ethanol (95%v/v by cold maceration process in which powdered drug material were soaked in ethanol for 24 hrs. The cycles were continued till complete recovery of all the dissolved plant materials. The extract were then concentrated in petridishes at room temperature and stored in a refrigerator in air tight bottles for further analysis. Similarly aqueous extract were obtained by cold maceration process in which powdered drug material were soaked in water for 24 hrs. Then the resulting filtrate concentrated to obtain a aqueous extracts of *Vitex nigundo linn, Azardirachta indica linn and seeds* of *trigonella foenum graecum.* The obtained extracts were subjected to systematic preliminary phytochemical screening after extraction with solvent ethanol and water. The extracts were subjected for phytochemical investigation by qualitative chemical tests.

Phytochemical Analysis:

Qualitative chemical tests for identifying various phytoconstituents present were carried out on ethanolic and water extracts of *Vitex nigundo linn, Azardirachta indica linn* and seeds of *trigonella foenum graecum* constituents are as follows**.**(6,7,8)

Table.1 Preliminary phytochemical screening of the Ethanolic extracts of plants.

Test	***Vitex nigundo* Linn Ethanol extract**	***Azadaricta indica* Ethanol extract**	***Trigonella foenum graecum* Ethanol extract**
Carbohydrate	-	-	-
Protein/amino acid	-	+	-
Fats/ waxes	-	+	-

Glycoside	-	+	-
Flavonoides	+	+	+
Alkaloids	-	+	-
Terpenes	+	+	+
Steroids	+	+	+
Saponins	-	+	-
Phenolics /Tannins	+	+	+
Volatile oil	+	-	+

+ (presence) - (Absence)

Table.2 Preliminary phytochemical screening of the Aqueous extracts of plants.

Test	***Vitex nigundo* Linn Aqueous extract**	***Azadaricta indica* Aqueous extract**	***Trigonella foenum graecum* Aqueous extract**
Carbohydrate	-	-	-
Protein/amino acid	-	+	-
Fats/ waxes	-	+	-
Glycoside	-	+	-
Flavonoides	+	+	+
Alkaloids	-	+	-
Terpenes	+	+	+
Steroids	+	+	+
Saponins	-	+	-
Phenolics /Tannins	+	+	+
Volatile oil	+	-	+

Fourier Transform Infrared Spectrophotometer (FTIR): The concentrated extracts of *Vitex nigundo linn, Azardirachta indica linn and* seeds of *trigonella foenum graecum* were used for FTIR analysis in Parkin Elmer. model no L1600401 Spectrum Two DTGS Serial Number 107435, UK with a scan range from 400 to 4000cm-1 as per instrument manual.

Result and Discussion :

The FTIR spectra for leaves of *Vitex nigundo linn, Azardirachta indica linn* and seeds of *trigonella foenum graecum* were prepared in Ethanol and aqueous extracts are represented in figure 1 to 6. The data in the table 1to 6 Show peak values present in the leaf extracts of *Vitex nigundo linn, Azardirachta indica linn and* seeds of *trigonella foenum graecum* Obtained by FTIR analysis and the probable functional Group. Following are the descriptions of results from FTIR spectra that could be interpreted for effective use of Plant extracts.

***Azardirachta indica* Aqueous Extracts:** exhibited characteristics band at 3248.11 cm^{-1} for O-H stretching, 1731.60 cm^{-1} for C=O carbonyl group, 1565.84 for C=C, 1396.37 cm^{-1} for tert-butyl CH_3 group and 1202.27 cm^{-1} for C-O stretching, 1035.09 for C-O stretching, 628.39 for C-Br stretch for Aliphatic bromo compounds, 569.87 for C-I stretch.

***Azardirachta indica* Ethanolic Extracts:** Showed characterstics band at 3307.68 cm^{-1} for O-H stretch, hydroxy group, H-bonded polyhydroxy compounds, 2919.01 cm^{-1} for C-H stretching, 2850.41 cm^{-1} for C-H stretching, 1714.39 cm^{-1} for C=O for carbonyl group, 1609.78 cm^{-1} for C=C, C=O stretching vibrations, Ketone group, 1031.68 for C-O, 817.22 cm^{-1} for S-OR, 516.17 cm^{-1} for sulfur functions disulfide groups (S-S).

***Trigonella foenum graecum* Aqueous extract:** Aqueous extract of fenugreek exhibit the characterstics band at 3392.32 cm^{-1} for O-H stretching of carboxylic acid, 2100.25 cm^{-1} for C≡ C bond of alkyne, 1647.77 $cm^{-1}$for for C=C stretch of alkene, 1492.44 cm^{-1} for C=C stretch of aromatic, 1321.27 cm^{-1} for C-N of amine, 1178.22 cm^{-1} for C-O stretch of alcohol, 1155.53 cm^{-1} for C-O stretch of alcohol.

***Trigonella foenum graecum* Ethanolic extract**: ethanolic extract showed characterstics band at 3009.57 cm^{-1} for C-H stretch of carboxylic acid, 2923.43cm^{-1} For C-H stretch of alkane, 2853.67 cm^{-1} for C-H stretch, 1709.24 cm^{-1} for C=O of ketone, 1458.43 cm^{-1} for C=C of alkenes, 1378.22 cm^{-1} for C=C blend of ch_2 group, 1178.40 cm^{-1} C-O of alcohol, 1046.02 $cm^{-1}$for C-O, 721.47 $cm^{-1}$for C-H of methyl group.

***Vitex nigundo* Aqueous extract:** exhibit the characterstics band at 3271.33 cm^{-1} for O-H alcohol H-bonded, 1593.81 cm^{-1} for C= C Aromatic stretch, 1386.98 $cm^{-1}$for for CH_3, 1273.30 cm^{-1} and 1031.35 cm^{-1} for C-O stretch of alcohol.

***Vitex nigundo* Ethanolic extract:** exhibited the characterstics band at 3306.84 cm^{-1} for C-H stretch hydroxy group H-bonded, 2918.04 cm^{-1} for O-H stretch, 1702.40 $cm^{-1}$for for C=O stretch carbonyl compound, 1608.15 cm^{-1} for C=O stretching vibration, ketone group and 1515.48 cm^{-1} for C=C-C Aromatic ring.

Table 1 *Azardirachta indica* Linn aqueous extract:

Peak Number	X (cm-1)		Y (%T)
1	3248.11	O-H	57.32
2	1731.60	C=O	88.93
3	1565.84	C-C	43.60
4	1396.37	CH_3	51.42
5	1202.27	C-O	72.10
6	1035.09	C-O	48.20
7	674.95	C-H	43.33
8	628.39	C-Br	38.38
9	569.87	C--I	33.72
10	554.89	unknown	35.81
11	534.64	unknown	31.25
12	522.95	unknown	30.80
13	518.72	unknown	31.04
14	506.94	unknown	34.00
15	497.05	unknown	36.52
16	487.04	unknown	36.54
17	471.03	unknown	28.32
18	459.17	unknown	27.71

Table 2: *Azardirachta indica* ethanol extract:

Peak Number	**X (cm-1)**	**Types of functional groups**	**Y (%T)**
1	3307.68	O-H	65.86
2	2919.01	C-H	73.23
3	2850.41	C-H	78.96
4	1714.39	C=O	80.75
5	1609.78	C=C	78.14
6	1366.68	C-O	70.45
7	1031.50	C-C	39.45
8	817.22	C=C	64.89
9	776.34	unknown	62.09
10	516.17	S-S	45.33

Table 3: ***Trigonella foenum graecum*** **Ethanolic extract:**

Peak Number	**X (cm-1)**	**Types of functional groups**	**Y (%T)**
1	3009.57	O-H	88.38
2	2923.43	C-H	63.62
3	2853.67	C-H	71.70
4	1709.24	C=O	66.90
5	1458.43	C=C	78.96
6	1378.22	C=C	81.44
7	1178.40	C-O	74.39

8	1046.02	C-O	73.42
9	721.47	C-H	73.44

Table 4: ***Trigonella foenum graecum*** **aqueous Extract:**

Peak Number	**X (cm-1)**	**Types of functional groups**	**Y (%T)**
1	3392.32	O-H	80.88
2	2100.25	C≡C	98.45
3	1647.77	C=C	92.54
4	1492.44	C=C	106.24
5	1321.27	C-N	121.23
6	1178.22	C-O	113.81
7	1155.53	C-O	114.58

Table 5: ***Vitex nigundo*** **ethanolic extract:**

Peak Number	**X (cm-1)**	**Types of functional groups**	**Y (%T)**
1	3306.84	O-H, stretch, Hydroxy group, H-bonded	69.95
2	2918.04	O-H stretch acidic,	72.28
3	1702.40	C=O stretch, carbonyl compound.	78.79
4	1608.15	C=O stretching vibration, ketone group	76.19
5	1515.48	C=C-C , Aromatic ring.	85.55
S6	1444.40	unknown	73.34
7	1372.42	unknown	70.90
8	1268.33	unknown	61.82

9	1164.48	unknown	66.08
10	1027.69	unknown	35.61
11	922.35	unknown	67.89
12	853.07	unknown	65.24
13	817.62	unknown	66.69
14	769.87	unknown	59.91
15	503.20	unknown	46.47

Table 6: ***Vitex nigundo*** **aqueous extract:**

Peak Number	**X (cm-1)**	**Types of functional groups**	**Y (%T)**
1	3271.33	O-H	54.97
2	1593.81	C=C	59.71
3	1386.98	CH_3	62.86
4	1270.30	C-O	64.69
5	1031.35	C-O	40.91

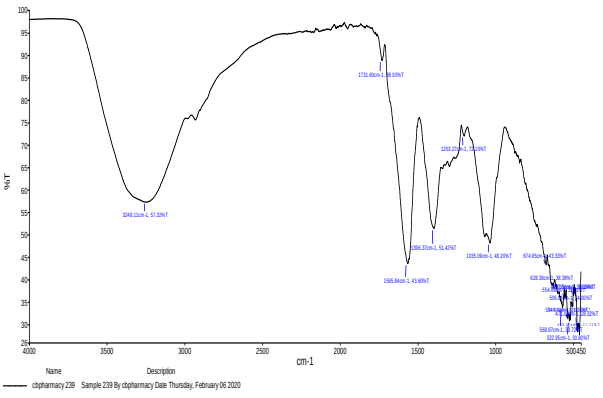
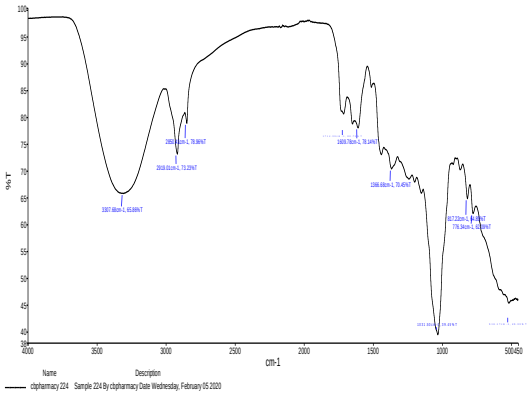


Figure: 1



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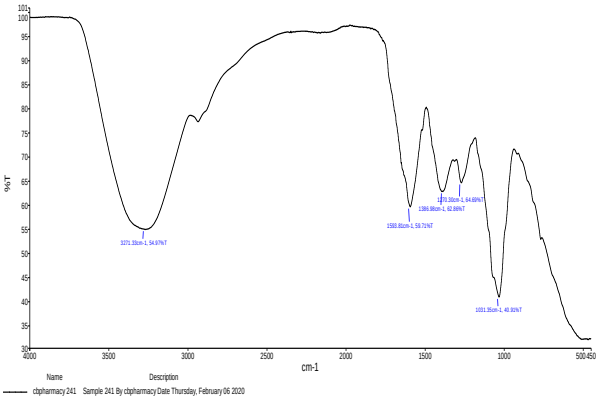

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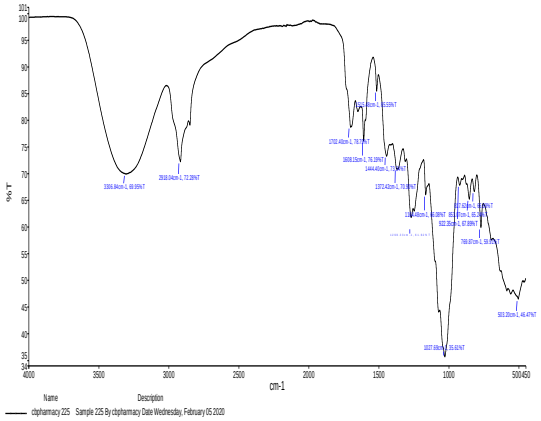

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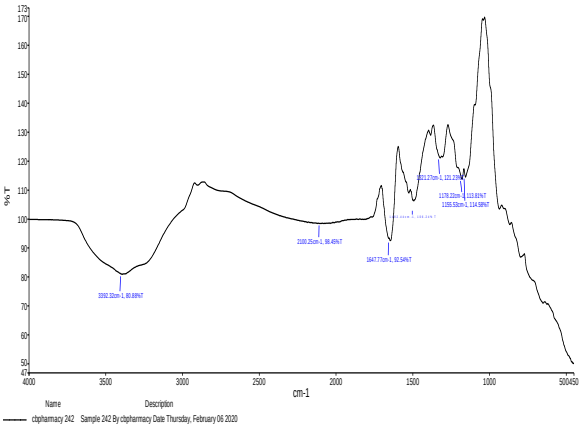

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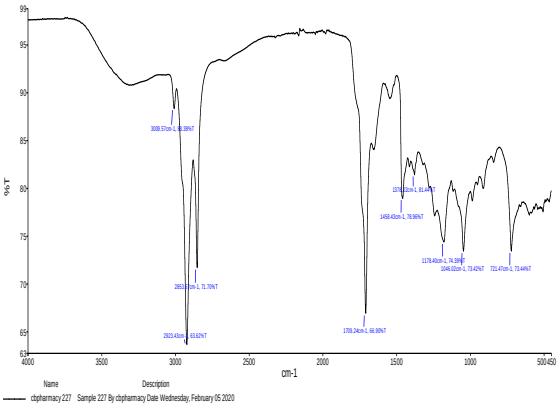

Figure 6.

extract **Figure 3:** FTIR spectra of *Vitex nigundo* aqueous extract **Figure 4:** FTIR spectra of *Trigonella foenum graecum* ethanol Extract: **Figure 5:** FTIR spectra of *Vitex nigundo* ethanol extract **Figure 6:** *Trigonella foenum graecum* aqueous Extract:

Conclusion:

From the above results obtained in this study, it is concluded that the leaf extracts (in water and ethanol) of plants *Vitex nigundo linn*, *Azardirachta indica linn* and *trigonella foenum graecum* Seed extracts with their phytoconstituents may act as an effective source for the treatment of microbes causing diseases. The different functional groups present in these plant extracts probably indicate the presence of phytoconstituents like carbohydrates, carotenoid, Flavonoids, amino acids, amides, starch, phosphates, lipids and phenols. Among the different functional groups observed in these plant extracts, OH group was found to be commonly observed in the ethanolic extracts of these four plants. As we know OH group present in those plant extract that possesses greater inhibitory activity against different microorganisms. So the selected plant extract possesses significant antioxidant and antimicrobial activity against disease causing pathogens.

References:

1. Ncube NS, Afolayan AJ and Okoh AI, Assessment techniques of antimicrobial properties of natural compounds of plant origin. Current methods and future trends. African J Biotechnology..(2008):7; 1797-1806.

2. VinothS,Rajeshkanna P Gurusaravanan P and JayabalanN,Evaluation of phytochemical, antimicrobial and GC- MS analysis of extracts of Indigoferatrita L.F . Spp. subulata (vahl ex poir) .IntJAgri Res(2011); 6(4) : 358-367.

3. P. Gans, Vibrating Molecules: an Introduction to theInterpretation of Infrared and Raman Spectra, Chapman & Hall, London, 1975.

4. Ragavendran,P., Sophia,D., Arul Raj,C., and Gopalakrishnan V.K., Functional group analysis of various extracts of Aervalanata (L.,) by FTIR spectrum. Pharmacologyonline. 1, 358-364. 2011.

5. R.Ashokkumar* and M. Ramaswamy, Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian Medicinal plants. Int.J.Curr.Microbiol.App.Sci (2014) 3(1): 395-406.

6. Khandelwal K. Practical pharmacognosy. Pragati Books Pvt. Ltd.; 2008 Sep 7, 18.15-18.18

7. Khandelwal K. Practical pharmacognosy. Pragati Books Pvt. Ltd.; 2008 Sep 7, 25.1-25.9