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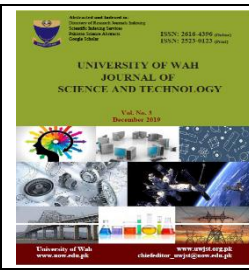

Phytochemical Screening by FTIR Spectroscopic Analysis of Leaf Extracts of Monotheca Buxifolia

Shahzad Ahmad, Faisal Nawaz, Shazia Naheed, Zaheer Ahmad and Tahir Mehmmod

Abstract -This study is aimed to analyze the extracts *n*-hexane, toluene, chloroform, carbontetra chloride, butanol, methanol by keeping in view the pharmacological importance of the leaves of *Monotheca buxifolia* A. DC. Fourier transform infrared (FTIR) spectroscopic studies concludes that the peak value characteristic indicate the presence of several functional groups of important bioactive compounds in the extracts. The FTIR analysis of *M. buxifolia* conforms the presence of amides, alcohols, phenols, alkanes, ketones, aldehydes, aromatic compounds and carboxylic acids using the peaks values of functional groups. The results of current study are produced by FTIR spectrum supporting the medicinal importance of plants such as *M. buxifolia that is* used as a source for folk medicine.

Index Terms*—FTIR analysis, Functional groups, Medicinal importance, Extraction, *M. Buxifolia

I. Introduction

IN ancient times the plants, their parts, or extracts played a vital role in maintenance, recovery of health and cure of the diseases. Thousands of years ago, North Africans, Chinese and Indian had written evidences related to the usage of plants as medicines and curing of many diseases.

Till the nineteenth century the scientists separated the active constituents from a variety of medicinal plants. Morphine was separated by a scientist Friedrich Serturner from plant *Papaver Somniferum* in 1806, and after that thousands of natural products isolated from the various plants that are using as medicine. Atropine was extracted from plant *Atropa belladonna*. Similarly, strychnine was extracted from natural source which is used as pesticide and ziconotide was obtained from a *cone snail (conus magus)*.

Recent study of World Health Organization (WHO) reveals that nearly 80% of the globe trusts on ancient medicines [1]. Nearly 120 medicines were prescribed in USA, which were extracted from natural resources. 90 out of 120 medicines were obtained directly or indirectly from plant sources [2]. About 46.9 percent of the drugs were used in the treatment of cancer in the market, all of them were obtained from natural resources. During the year 1981 to 2006 almost hundred anticancer agents were discovered, out of them twenty five (25) were obtained from natural product, while the remaining eighteen (18) derivatives were mimics, eleven (11) were the natural products pharmacophore and nine (9) were pure natural products [3].

Functional groups and shape of molecules are responsible for the medicinal properties of bioactive compounds present in medicinal plants. It had been discovered during the study of fifteen (15) medicinal plants that were studied *in vitro* efficacy of bioactive compounds against ESβL- producing multi drug resistance bacteria. In 2006, the major functional groups from extract of four medicinally important plants *via* IR spectroscopy were identified [4]. Saponins were detected in crude dry powder from the extract of eleven (11) plants using FTIR spectroscopy [5]. Using FTIR spectroscopic technique, samples of powder of leaf, stem and roots of *Eclipta alba* and *Eclipta Prostrate* were analyzed by [6]. Through FTIR analysis, Ramamoorthi and kannan partitioned the bioactive collections in the *Calotropis gigantean* dry leave's extract in 2007 [7]. The FTIR study had been done on aqueous methanolic leaf extract of *bauhinia racemosa* and its phytochemical study was done by Gaurav kumar and co-workers in 2010 [8]. Presence of functional groups in different extracts of SEA *Aerva lanata* were reported by by means of spectroscopic methods 2011 [9]. Elements and functional groups were reported by FTIR spectroscopic method for the extract of whole plant of *Lchnocarpus frutescens* [10].

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Literature survey on important medicinal plant *M. buxifolia* indicates that the plant parts were not subjected for the FTIR spectroscopic analysis and work was not completed on functional group analysis. Therefore, present research has been done for functional group analysis of phytochemical compounds which were present in plant *M. buxifolia* leaves extracts (in different solvents according to their escalating polarity) by FTIR analysis [11-20]. Against diseases the most using plant part are leaves. Fruit of M. buxifoia is traditionally or locally used as medicine such as purgative, hematenic, laxative, vermicide, antipyretic, anti-nociceptive, anti-inflammatory activities, gastritis and most important in the management of urinary tract infections (UTI'S) and sometime eye infections as well [21-22].

II. Material and Method

A. Collection of plant

Fresh leaves of medicinal plant *Monotheca buxifolia*, weighing 12.5 kg, were collected from Akhori village and Kala Chita hills district Attock in August 2016 for analysis purpose.

B. Preparation of leaf extract

The leaves of plant were soaked and washed with fresh water to remove the dust particles and vice versa. Leaves were scattered under shade and dried at room temperature (23ºC) [11]. When they were completely dried and weighed again. The plant leaves were dipped into different solvents according to their escalating polarity (n-hexane 0.1< carbon tetra chloride 1.56< toluene 2.4 < ethyl acetate 2.8 < butanol 3.9 <chloroform 4.1< methanol 5.1) and kept for 20 days under room temperature as shown in Table I. The extract of leaves was filtered by using Whitman No.1 filter paper. The extract was pooled, evaporated and concentrated *via* rotary evaporator and kept in closed bottles for additional analysis.

C. Fourier Transform Infrared Spectrophotometer (FTIR) Analysis

FTIR is most powerful for identification of functional groups and to check the presence of chemical bonds that are present in a compound. Each bond vibrating with its characteristic frequency which depend upon the strength of bond as well as masses of bonded atoms. Frequency of the electromagnetic radiations falling in the infrared region (2.5-16 m or 4000-625 cm) in the electromagnetic spectrum corresponds to the frequency of the most molecular vibrations.

The wavelength of light captivated is distinctive of chemical bond as can been seen by annotated spectrum. Information obtained from IR spectra interpretation; presence and environment of functional groups, especially those containing X-H or C-X type bonds such as O-H, C=O, N-H, C=C, C-H, CΞC, C-C, and CΞN can be determined.

Dried extracts of different solvents were used for FTIR analysis, whereas samples were collected in bottles. The sample of each solvent extract was loaded in FTIR spectroscope (BRUKER Model ALPHA FTIR spectrophotometer) with a scan range from 400 to 4000 cm^{-1}.

TABLE I
LEAVES EXTRACT DETAIL OF PLANT MONOTHECA BUXIFOLIA

S. No	Solvents	Quantity of solvent used (liter)	Code	Weigh of extract (g)
01	N –hexane	10	SAA-001	30
02	Carbon tetra chloride	l.25	SAA-002	1.4
03	Toluene	l.25	SAA-003	1.8
04	Butanol	l.25	SAA-006	1.9
05	Chloroform	l.25	SAA-007	2.6
06	Ethyl acetate	l.25	SAA-005	4.1
07	Methanol	5	SAA-004	80.6

III. Results and Discussion

The FTIR spectrum of leaves extracts of plant *M. buxifolia* in different solvents are given in *Fig. 1-7*. The interpreted peak values and probable functional groups present in the leaf extracts (obtained by FTIR analysis) are shown in the Table I - VI.

A. FTIR Spectral data Interpretation n-Hexane (n-H)/Butanol (BT) Extract

n-H/BT extracts of *M. buxifolia* depicted same spectra, a characteristic band at 1735 cm^{-1} as shown in *Fig. 1 and Fig. 4* indicating the presence of carbonyl compounds (C=O) such as aldehydes, ketones, etc. and at 2920 cm^{-1} for C-H group. A peak at 2850 cm^{-1} for $–CH_2$ symmetric stretching, and a peak at 1460 cm^{-1} for $–CH_3$ anti sym str and 1376 cm^{-1} sym def of C-H group of alkanes as given in Table II.

B. Carbon tetra chloride (CTC) Extract

CTC extract of *M. buxifolia* depicted a characteristic band in FTIR spectra at 1735 cm^{-1} as shown in *Fig. 2* indicating the presence of carbonyl compounds (C=O) such as aldehydes, ketones, esters, amides etc and at 2916 cm^{-1} for C-H group. A peak at 2849 cm^{-1} for $–CH_2$ symmetric stretching, a peak at 1446 cm^{-1} for $–CH_3$ anti sym str and 1378 cm^{-1} sym def of C-H group of alkanes. A number of peaks at 1240 cm^{-1}, 1205 cm^{-1}, 1168 cm^{-1}, 1148 cm^{-1}, 1092 cm^{-1}, 1050 cm^{-1}, 1028 cm^{-1}, showing C-O str may be for C-O-C group from anhydride or C-O str for pri, sec or teriary alcohols. A peak at 971 cm^{-1} –OH out of plane def for AcO-H, free H-bonding and various peaks at 882 cm^{-1}, 861 cm^{-1} and 801 cm^{-1} CH out of plane Ar-H m-disubstituted for aromatic compound as given in Table III.

C. Toluene/ Ethyl acetate (EA) Extract

Toluene/EA extract of *M. buxifolia* depicted same spectra tremendously, a characteristic band at 1687 cm^{-1} as shown in *Fig. 3 and Fig. 6* representing the presence of a-b unsaturated C=O group ketonic compound and at 2916

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cm^{-1} for C-H group. A peak at 2848 cm^{-1} for $–CH_2$ symmetric stretching for alkanes, and a peak at 1460 cm^{-1} for $–CH_3$ anti symmetric stretching and 1377 cm^{-1} sym def of C-H group of alkanes. A peak on 996 cm^{-1} -OH out of plane def for AcO-H, free H-bonding. A peak at 719 cm^{-1} showing $–CH_2$ rocking vibration for alkanes as given in Table IV.

D. Chloroform (CF) Extract:

CF extract of *M. buxifolia* depicted a characteristic band in spectra at 1687 cm^{-1} as shown in *Fig. 5* indicating he presence of a-b unsaturated C=O group ketonic compound and at 2916 cm^{-1} for C-H group. A peak at 2848 cm^{-1} for $–CH_2$ symmetric stretching for alkanes, and a peak at 1461 cm^{-1} for $-CH_2$ scissoring. A peak at 996 cm^{-1} $–CH_2$ out of plane for alkanes and a peak at 973cm^{-1} for –OH out of plane def for AcO-H, free H-bonding and a peak at 712 cm^{-1} for $-CH_2$ rocking as given in Table V.

E. Methanol (ME) Extract:

Methanol extract of *M. buxifolia* depicted a characteristic band in spectra at 3292 cm^{-1} very broad peak as shown in *Fig. 7* indicating -OH functional group, OH str for alcohols or phenols. A band at 1616 cm^{-1} indicating he presence of carbonyl compounds (C=O) such as aldehydes, ketones, etc and at 2918 cm^{-1} for C-H group. A peak at 1448 cm^{-1} for $–CH_3$ anti-symmetric stretching vibration and 1376 cm^{-1} sym def of C-H group of alkanes. Two peaks at 1394 cm^{-1} and 1318 cm^{-1} indicating -OH in plane def for ter and sec alcohols. A peak at 1271 cm^{-1} indicating alkyl-aryl Ar-O str and a peak at 1205 cm^{-1} for phenols. A number of peaks at 1068 and 1023 cm^{-1} showing C-O str of C-O-C group from anhydride and 971 cm^{-1} –OH out of plane def for AcO-H, free H-bonding and a band on 812 cm^{-1} for CH out of plane as given in Table VI.

Calotropis gigantea leaves and latex have cardiac glycosides, which were identified as calotropin and calotropogenin [23]. Same results are found as mentioned earlier and also reported the presence of organic compounds such as amino acids, chlorophyll, amides, lignin's, carbohydrates and starch in *Calrops gigantean* plant [7].

TABLE II
FTIR ANALYSIS AND PEAK VALUES OF DIFFERENT FUNCTIONAL GROUPS OBTAINED FROM *N*-HEXANE AND BUTANOL LEAVES EXTRACT OF *M. BUXIFOLIA*

Type of solvent	Peak values cm^{-1}	Functional groups
n-Hexane/Butanol	2919	C-H stretching
	2850	$-CH_2$ sym str
	1735	C=O carbonyl compound
	1460	$-CH_3$ antisym str
	1376	-C-H bending

TABLE III
FTIR ANALYSIS AND PEAK VALUES OF DIFFERENT FUNCTIONAL GROUPS OBTAINED FROM CARBON TETRA CHLORIDE LEAVES EXTRACT OF *M. BUXIFOLIA*

Type of solvent	Peak values cm^{-1}	Functional groups
Carbon tetrachloride	2916	C-H stretching
	2849	$-CH_2$ sym str
	1735	C=O carbonyl compound
	1446	$-CH_3$anti sym str
	1378	C-H group
	1168	C-O str of C-O-C for
	1092	acid anhydride
	1205	Phenol
	1148	Tertiary alcohol
	1050	Secondary alcohol
	973	-OH out of plane
	861	CH out of plane for AR-H
	801	m-disub.

TABLE IV
FTIR ANALYSIS AND PEAK VALUES OF DIFFERENT FUNCTIONAL GROUPS OBTAINED FROM TOLUENE/ ETHYL ACETATE LEAVES EXTRACT OF *M. BUXIFOLIA*

Type of solvent	Peak values cm^{-1}	Functional groups
Toluene/ Ethyl acetate	2916	C-H stretching
	2848	$-CH_2$ sym str
	1687	C=O group of amide or α,β-unsaturated ketone
	1460	$-CH_3$ anti sym str
	1377	-C-H group
	719	$-CH_2$ rocking

TABLE V
FTIR ANALYSIS AND PEAK VALUES OF DIFFERENT FUNCTIONAL GROUPS OBTAINED FROM CHLOROFORM LEAVES EXTRACT OF M. BUXIFOLIA

Type of solvent	Peak values cm^{-1}	Functional groups
Chloroform	2916	C-H stretching
	2848	-CH2 sym str
	1687	C=O α,β-unsaturated ketone
	1461	-CH3 anti sym str
	1254	Ar-O str
	1073	C-O Str
	1030	R-O str
	996	-OH out of plane for AcO-
	973	H, free H bonding
	712	-CH2 rocking

TABLE VI
FTIR ANALYSIS AND PEAK VALUES OF DIFFERENT FUNCTIONAL GROUPS OBTAINED FROM METHANOL LEAVES EXTRACT OF M. BUXIFOLIA

Type of solvent	Peak values cm^{-1}	Functional groups
Methanol		O-H group
	3292	C-H stretching
	2918	C=O carbonyl compound
	1616	-CH3 anti sym str
	1448	-OH in plane def or
	1394	secondary and Primary
	1318	alcohol
	902	-OH out of plane
	812	CH out of plane for AR-H
		m-disubst

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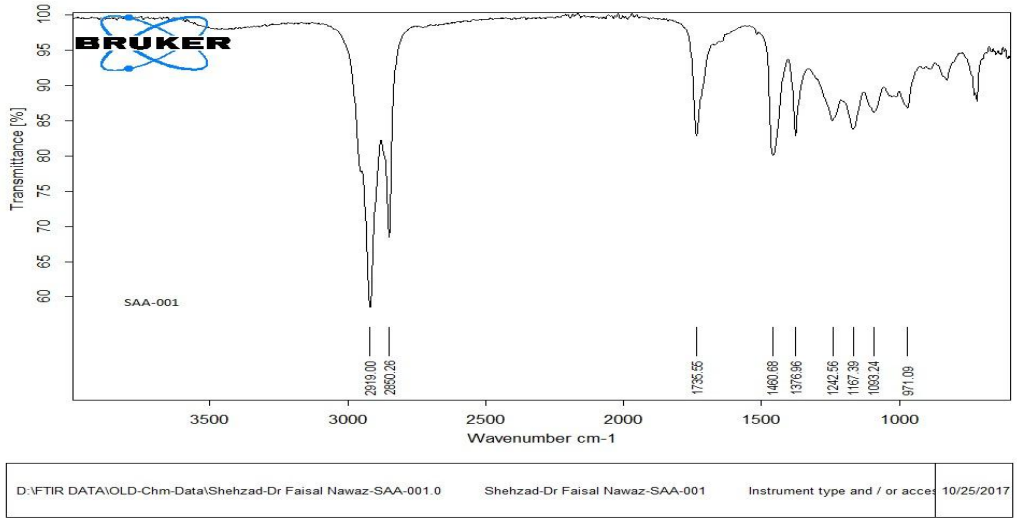

Fig. 1. FTIR Spectra of N-hexane extract of leaves of M.buxifolia.

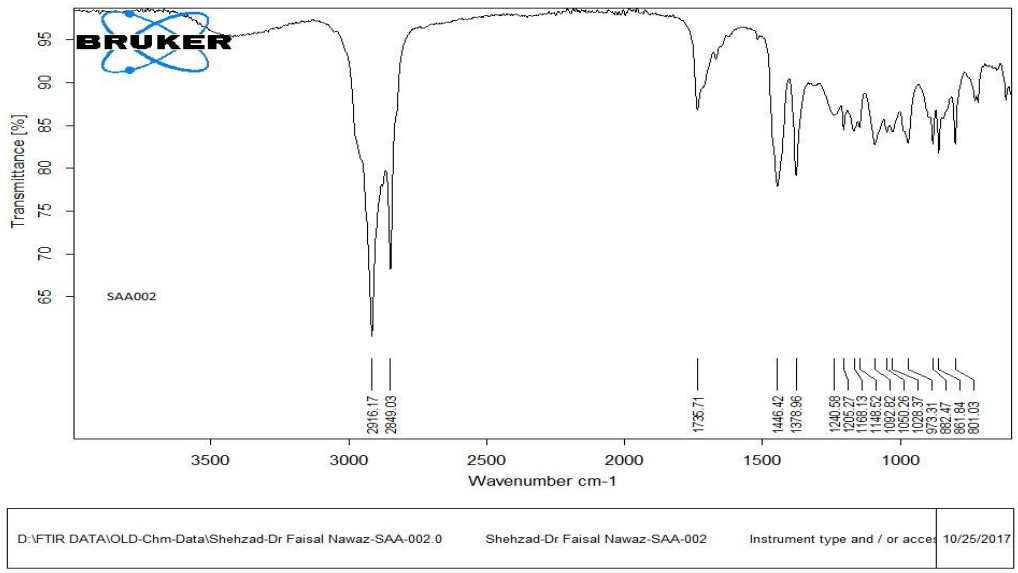

Fig. 2. FTIR Spectra of CCl4 extract of leaves of M.buxifolia.

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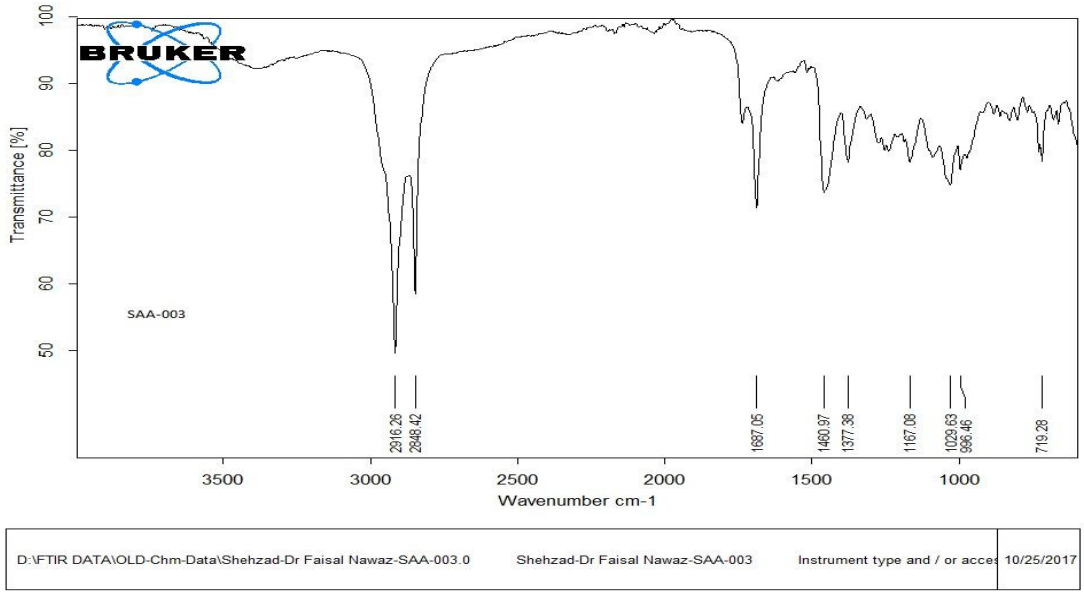

Fig. 3. FTIR Spectra of toluene extract of leaves of M.buxifolia.

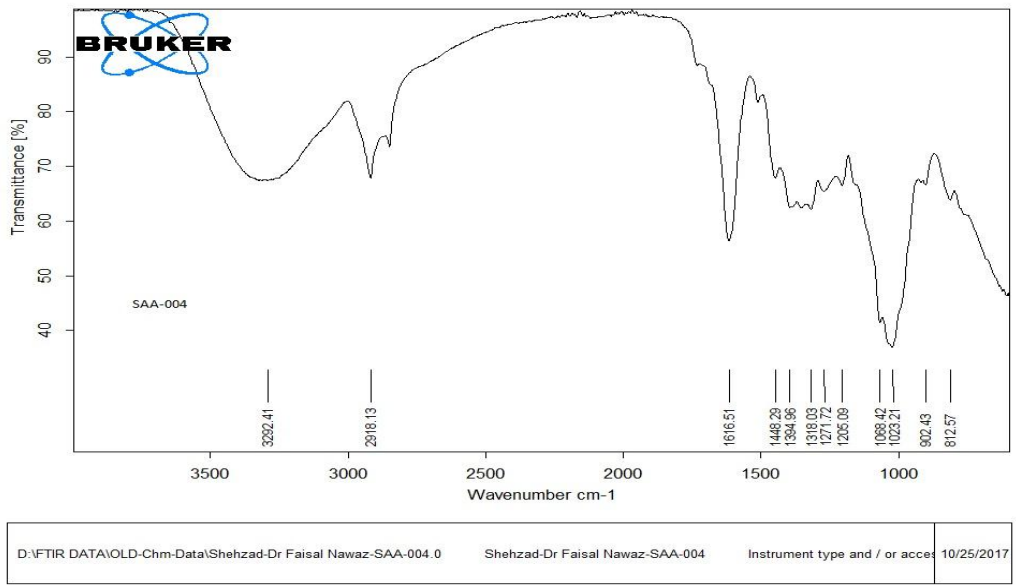

Fig. 4. FTIR Spectra of butanol extract of leaves of M.buxifolia.

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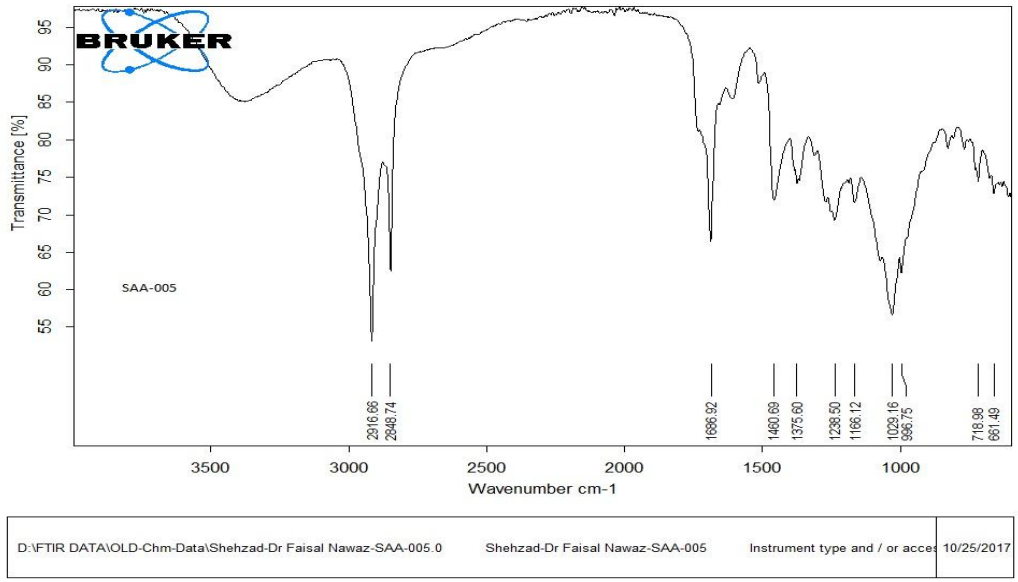

Fig. 5. FTIR Spectra of chlorofoarm extract of leaves of M.buxifolia.

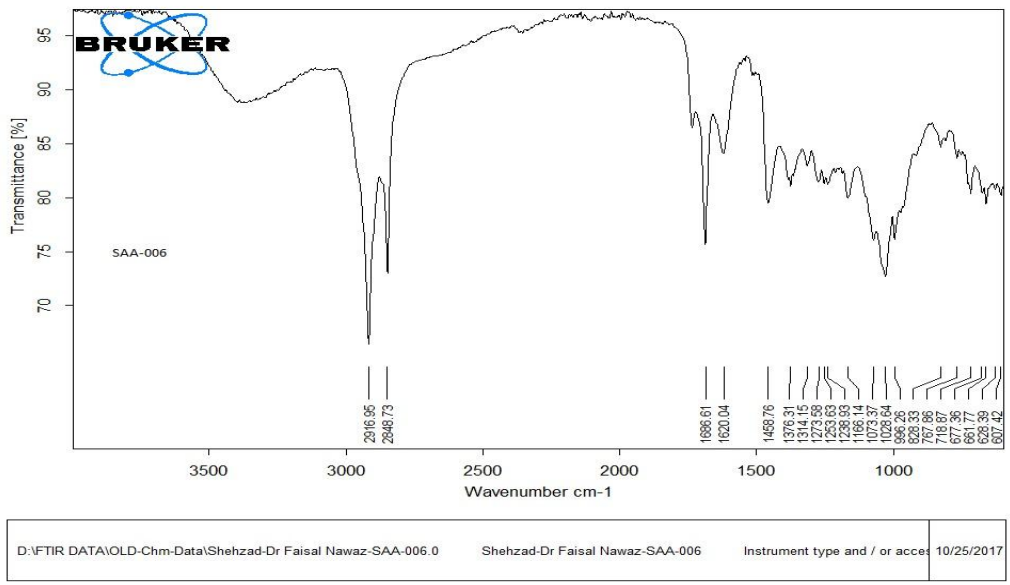

Fig. 6. FTIR Spectra of ethyl acetate extract of leaves of M.buxifolia.

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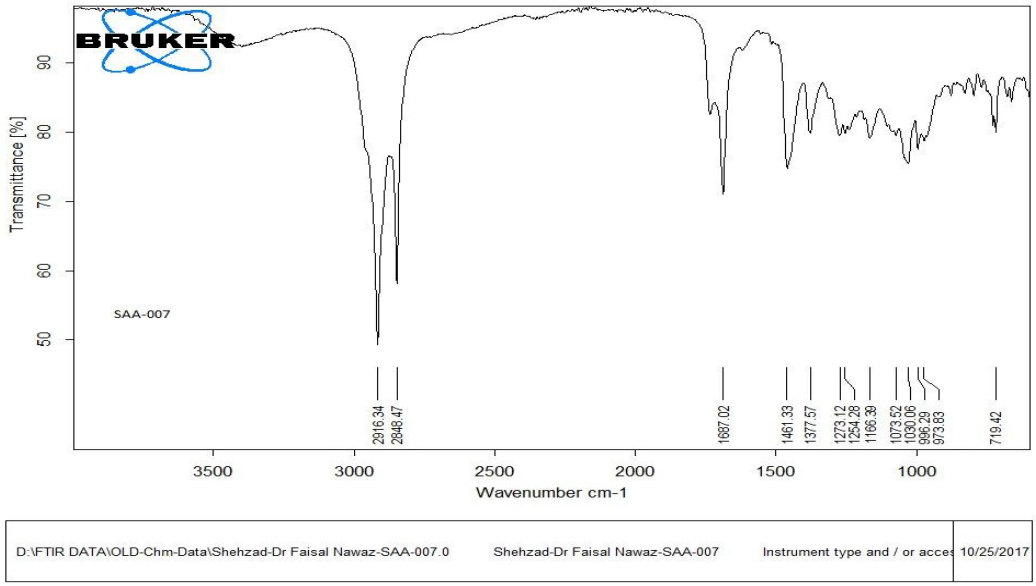

Fig. 7. FTIR Spectra oJf methanol extract of leaves of M.buxifolia.

FTIR and Energy Dispersive X-Ray Spectroscopy (EDS) spectra analysis of leaf, stem and roots of *Eclipta alba* and *Eclipta prostrate* was studied in [7] and various characteristic functional groups of amines, supher derivatives, carboxylic acids, amides, nitrates, carbohydrates, chlorated and polysaccharides are documented which are responsible for the medicinal use of both plants as herbal medicine. Useful elements like Zn, Ca, Mg, K, Cu, Na and Fe are present in higher concentration in *Eclipta alba* as compared to *Eclipta prostrate* [6]. Methanolic and aqueous leaf extracts of *Bauhinia racemosa* plant FTIR spectra interpretation shows that presence of fates, flavonoids, proteins, phenolic compounds, fats, carbohydrates tannins and saponins as major functional group [8].

Medicinal use of *Aerva lanata* in various disorders contains various medicinal properties due to the presence of various characteristic functional groups of organic hydrocarbons, amines, sulpher derivatives, carboxylic acids, amides, nitrates, carbohydrates, chlorates and polysaccharides. The presence of these functional groups were found in 2011 through FTIR analysis [9].

During the ethanolic extracts studies of *Ichnocarpus frutescens*, FTIR spectra revealed the different functional groups of carbonyl compounds, halogens, carboxylic acids, amines, organic hydro carbons, amides and amino acids [10]. Many other chemists, researcher and scientists worked on the identification of functional groups and reported the medicinal plants as folk medicines.

IV. Conclusion

The *M. buxifolia* is known for *its medincinal* properties. A comparative study has been conducted among different solvent (N-hexane, CCl4, Toluene, Butanol, Chloroform, Ethyl acetate, Methanol) extraction for screening of phytochemicals from *M. buxifolia* leaves. FTIR spectroscopic studies concludes that characteristic peak values indicates the several functional groups of important bioactive compounds that are detected in the extracts. The numerous functional groups detected in the altered extracts probably indicating the presence of Poly-phenolic and flavonoid compounds, carbohydrates, carotenoids, amino acids, lipids, glycogens, cellulose, amides, phosphates and starch etc.

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