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Original Article

QUALITATIVE PHYTOCHEMICAL SCREENING AND FTIR SPECTROSCOPIC ANALYSIS OF *GREWIA TILIFOLIA* (VAHL) LEAF EXTRACTS

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ABSTRACT

Objective: The present study aim is to analyse the phytochemicals present in *Grewia tilifolia* (Vahl) leaf extracts by using Qualitative phytochemical analysis, Fourier-transform infrared spectroscopy (FTIR).

Methods: The leaf extracts were prepared using eight different solvents. The phytochemical analysis Fourier transform infrared spectroscopy (FT-IR) analysis were performed using standard methods.

Results: The FTIR spectroscopic studies revealed different characteristic peak values with various functional groups present in the compounds of respective extracts. The FT-IR analysis shows the presence of different functional groups such as alcohols, phenols, alkanes, carboxylic acids, aldehydes, ketones, alkenes, primary amines, aromatics, esters, ethers, alkyl halides and aliphatic amine compounds, which showed major compounds present in the leaf extracts. The present study generated the FTIR spectrum profile for the medicinally important plant *Grewia tilifolia.*

Conclusion: The present study provides evediences that different extracts of *Grewia tilifolia* leaf is useful to cure many serious diseases which remained still problematic and for further isolation of bioactive compounds from the plant which could be of interest for the development of the new drug.

Keywords: *Grewia tiliaefolia* (Vahl), Phytochemicals, Fourier-transform infrared spectroscopy (FTIR)

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INTRODUCTION

Plants are a very useful source of various bioactive compounds which have direct or indirect use in the treatment of various human ailments [1]. A large number of medicinal plants are used as an alternative medicine for diseases of man and other animals since most of them are without side effects when compared with synthetic drugs. These are not only used as medicines to maintain their health care, also consumed as food by several Tribes of the Indian subcontinent. Phytochemicals are responsible for medicinal activities of the plants [2].

Based on this fundamental knowledge several pharmaceutical industries are established. The phytochemical constituents that are playing a significant role in medicines can be identified using crude extracts/drugs of the plants [3]. In current year's research in medicinal plants growing rapidly due to high pharmacological activities. Spectroscopic methods are very rapid and cost-effective than other conventional methods.

Fourier-transform infrared spectroscopy (FTIR) is a high-resolution analytical technique to identify the bioactive compounds based on the functional group present in them and to reveal the structure of the compounds [4, 5]. In Fourier-transform infrared spectroscopy (FTIR) molecules show absorption in a charecteristic range of frequency. The organic compounds mainly absorbed in the range of 4000-400 cm^{-1} which play a key role in the identification and charecterisation of the compounds which are present in the respective extracts [6].

***G**rewia tiliaefolia* belongs to the family Tiliaceae and it is a medium-sized tree, up to 20 m in height, with a clear bole and grey to blackish brown rough fibrous bark peeling off in thin flakes; leaves simple, alternate [7]. The flowers are yellow, small on thick axillary peduncles and fruits are globose drupes of the size of a pea, 2-4 lobed, black when ripe, seeds 1-2 [8]. The bark is astringent, sweet, acrid, refrigerant, oleaginous, expectorant, antipruritic, vulnerary, constipating, emetic, styptic, aphrodisiac and tonic [9]. The bark of the G. tiliaefolia showed the presence of three tri-terpenoids, viz. Betulin, Friedelin and Lupeol. Roots showed the presence of Friedelin and Lupeol [10]. Tri-terpenoids isolated from G. tiliaefolia bark at higher concentrations exhibited cytotoxic activity against LEUK-L1210 cells [11]. Stem bark of G. tiliaefolia showed the semen coagulant and cardiovascular effects [12]. It is useful in vitiated conditions of pitta and kapha, burning sensation, hyperdipsia, rhinopathy, ulcers, skin diseases, haematemesis and general debility. So, considering the clinical importance, the present study was designed to evaluate the standardised quality control of *grewia tilifolia* leaf [13].

Plant description

Large trees, bark dark brown or greyish-brown, rough, vertically striated; blaze brownish-red, fibrous, streaked; branchlets stellate-tomentose. Leaves simple, alternate; stipules 7-10 mm, lateral, auricled; petiole 8-35 mm, stout, swollen tipped, pubescent; lamina 6-36 x 3-24 cm, broadly ovate or obliquely ovate to round, base obliquely cordate or subcordate, apex acute, margin double serrate or crenate-serrate, glabrescent above and hoary pubescent beneath, coriaceous, 5-7-ribbed from base, prominent, lateral nerves 3-6 pairs, pinnate, prominent, intercostae scalariform, prominent. Flowers bisexual, yellow, in axillary umbels; peduncle 1.5-2 cm long; sepals 5, pubescent; petals 5, yellow, half the length of sepals, entire or notched, densely tomentose outside; stamens many, free, inserted on a glandular torus; gland densely villous on the margin; ovary superior, globose, hirsute, 2-4-celled, ovules 2-many; style subulate; stigma obscurely lobed, recurved. Fruit a drupe, globose to subglobose, reddish-purple, 2-lobed, sparsely hairy [14].

Grewia tiliaefolia Vahl, belongs to the family tilaceae is generally found in the forest of Anangan mala, which is a part of Western Ghats, in the northern end of Palakkad district, Kerala, India. *Grewia tiliaefolia* contain chemicals like D-erythro-2-hexenoic acid γ-lactone, Gulonic acid γlactone, Betulin, Friedelin, Lupeol, Tannins, Flavonoids, Hemicelluloses, Phenolics, Lupenol, and Lignin [15-18].

Related studies done in various plants as a reference like Iqbal Ahamad *et al.* (2006) detected major groups of compounds as the most active fraction of four plants extracts by infrared spectroscopy [19]. Ramamoorthi and Kannan (2007) screened the bioactive group

of chemicals in the dry leaf powder of *Calotropis gigantea* by FTIR analysis. Kareru *et al.* (2008) detected saponins in the crude dry powder of 11 plants using FTIR spectroscopy [20]. Analgesic and antipyretic activity of aqueous extract of Grewia tiliaefolia Vahl leaves were reported [21]. Antioxidant and antiproliferative activity of methanolic extract of Grewia tiliaefolia (Vahl) bark in different cancer cell lines [22]. The Fourier Transform Infrared (FTIR) spectroscopy allows the analysis of a relevant amount of compositional and structural information in plants. Moreover, FTIR spectroscopy is an established time-saving method to characterize and identified functional groups [23].

MATERIALS AND METHODS

Collection, authentication and processing of plant material

The plant material was collected in 2016 from kambalakonda forest area, Andhra Pradesh, India and authenticated by Dr. B. S. Padal, taxonomist, Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh. The Voucher specimens A. U.(B. D. H),NO.22231 were deposited in the herbarium, A. U. College of Pharmaceutical Sciences, Andhra University.

The samples were washed thoroughly in running tap water to remove soil particles and adhered debris and finally washed with sterile distilled water. The leaves were cut, shade dried, ground into a fine powder and stored in airtight polythene bags until use.

Extraction

The shaded dried leafs were powdered in the medical grinder. 100 grams of leaf powder was weighed, 300 ml of different solvents (hexane, petroleum ether, chloroform, ethyl acetate, methanol, acetone, benzene and distilled water) used each and individually with soxhlet extraction method for 72 h. The extract was filtered using Whatman No.1 filter paper and the filtrate was collected (crude extracts). It was then transferred to glass vials and kept at 4 °C for future use.

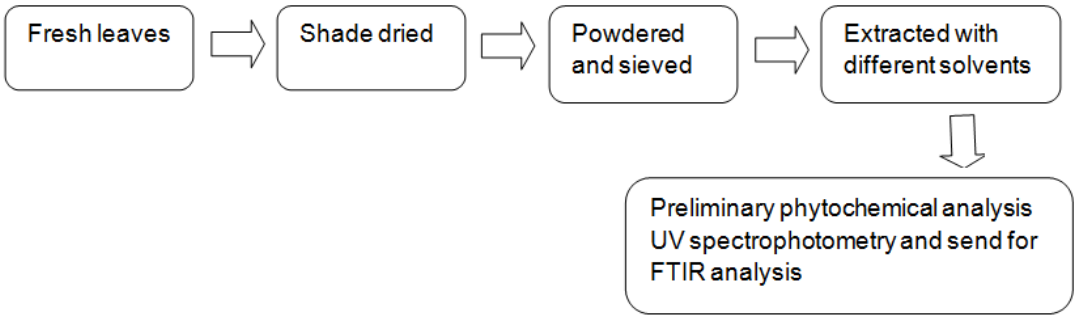

Fig. 1: Schematic representation of extraction processes

Preliminary phytochemical analysis

Qualitative phytochemical analysis was carried out [24-26] and the results observed were based on the colour change or precipitate formation after the addition of specific reagents.

Fourier transform infrared spectrophotometer (FTIR)

Fourier Transform Infrared Spectrophotometer (FTIR) is the most powerful tool for identifying the types of chemical bonds and functional groups present in compounds. The wavelength of light absorbed is characteristic of the chemical bond. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried powder of different solvent extracts of each plant materials was used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each plant specimen was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a Scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

RESULTS

Table 1: **Preliminary phytochemical screening**

Phytochemicals	Hexane	Ethyl acetate	Methanol	Crude methanol	Crude aqueous extract
Phytosterols	+	+	+	+	+
Terpenoids	+	+	+	+	+
Saponins	-	+	+	+	+
Flavonoids	-	+	+	+	+
Tannins	+	+	+	+	+
Carbohydrates	-	-	-	-	-
Alkalods	-	+	+	+	-
Amino Acids	-	-	-	-	-
Phenols	+	+	+	+	+
Quinones	-	-	+	+	-
Glycosides	-		+	+	+
Proteins	-	-	-	-	-

Successively extracted using Hexane; Ethylacetate; Methanol and Crude Methanol; Crude Aqueous extracts'+' indicates presence; '-' indicates an absence

Functional groups identification

The FTIR spectrum was used to identify the functional groups of the active components present in extract based on the peaks values in the region of IR radiation.

When the extract was passed into the FTIR, the functional groups of the components were separated based on the ratio of its peak. The results of FTIR analysis confirmed the presence of alcohol, phenol, alkanes, aldehyde, aromatic compound, secondary alcohol, aromatic amines, and halogen compound.

Table 2: Hexane extracts FTIR interpretation of compounds

Extract	Wavenumber (cm^{-1})	Functional group	Phytocompounds identified
Hexane	3741.33	Non bonded,OHstretching	Hydroxy group
	2922.81	Symmetric stretching of–CH_2(CH_2) vibration	Lipids,proteins
	2855.21	Symmetric stretching of–CH_2(CH_2) vibration	Lipids,proteins
	2376.61	O-H stretching	Carboxilic acid
	1734.32	C=O stretching	Aldehyde Compound
	1551.25	C=C stretching	
	1456.92	C=C-C Aromatic ring stretching	Aromatic Compound
	1377.55	O-H bening,alcoholic group	Phenol or tertiary alcohol
	1247.13	CN stretching	Aromatic primary amine
	729.25	C-Cl stretching	Aliphatic Chloro Compound

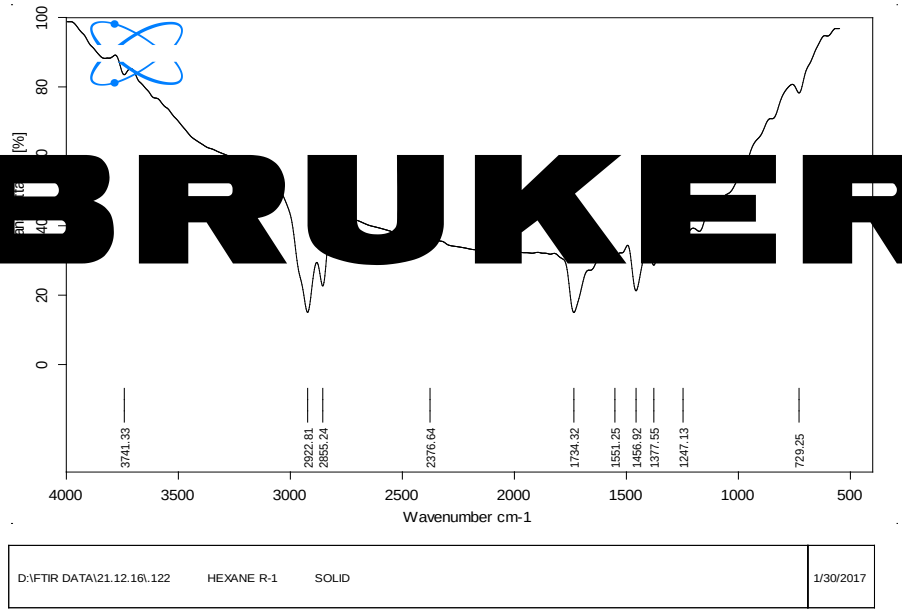

Fig. 1: FTIR spectrum of hexane extracts of *Grewia tilifolia* leaf

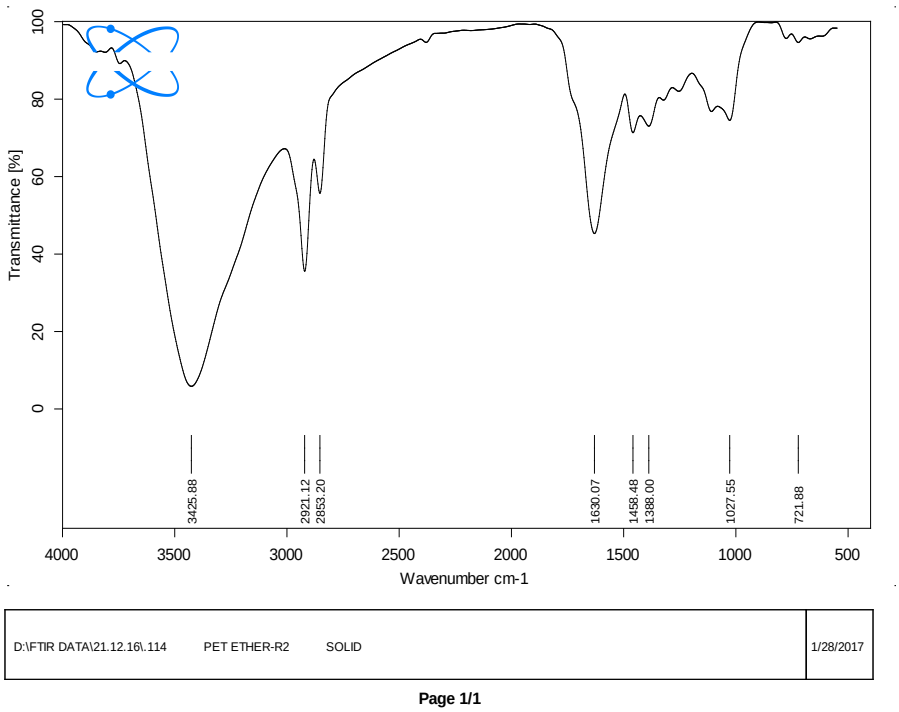

Fig. 2: FTIR spectrum of petroleum ether extracts of *Grewia tilifolia* leaf

Table 3: Petrolium ether extract FTIR interpretation of compounds

Extract	Wavenumber (cm^{-1})	Functional group	Phytocompounds identified
Petrolium Ether	3425.88	O-H stretching,H-bonded	Hydroxy Compound
	2921.12	Asymmetric stretching of–CH(CH_2) vibration	Saturated aliphatic Compound-Lipids
	2853.20	Symmetric stretching of–CH_2(CH_2) vibration	Lipids,proteins
	1630.07	C=O stretching	Ketone Compound
	1458.48	C=C-C Aromatic ring stretching	Aromatic Compound
	1388.00	O-H bening,alcoholic group	Phenol or tertiary alcohol
	1027.55	Phosphate ion	Phosphate Compound
	721.88	C-Cl stretching	Aliphatic Chloro Compound

Table 4: Chloroform extract FTIR interpretation of compounds

Extract	Wavenumber (cm^{-1})	Functional group	Phytocompounds identified
Chloroform	3807.14	Non bonded,Ohstretching	Hydroxy group
	3760.15	Non bonded,Ohstretching	Hydroxy group
	3612.15	Non bonded,Ohstretching	Hydroxy group
	2975.32	C-Hstretching	
	2866.14	Symmetric stretching of–CH_2(CH_2) vibration	Lipids,proteins
	2377.41	O-H stretching,Acidic	Carboxilic acids
	2275.21	Multiple bonding	Nitrile Compound
	1705.07	C=O stretching	Carbonyl Compound
	1652.78	C=Ostretching	Amide
	1547.80	C=C stretching	Aromatic
	1462.07	C=C-C Aromatic ring stretching	Aromatic Compound

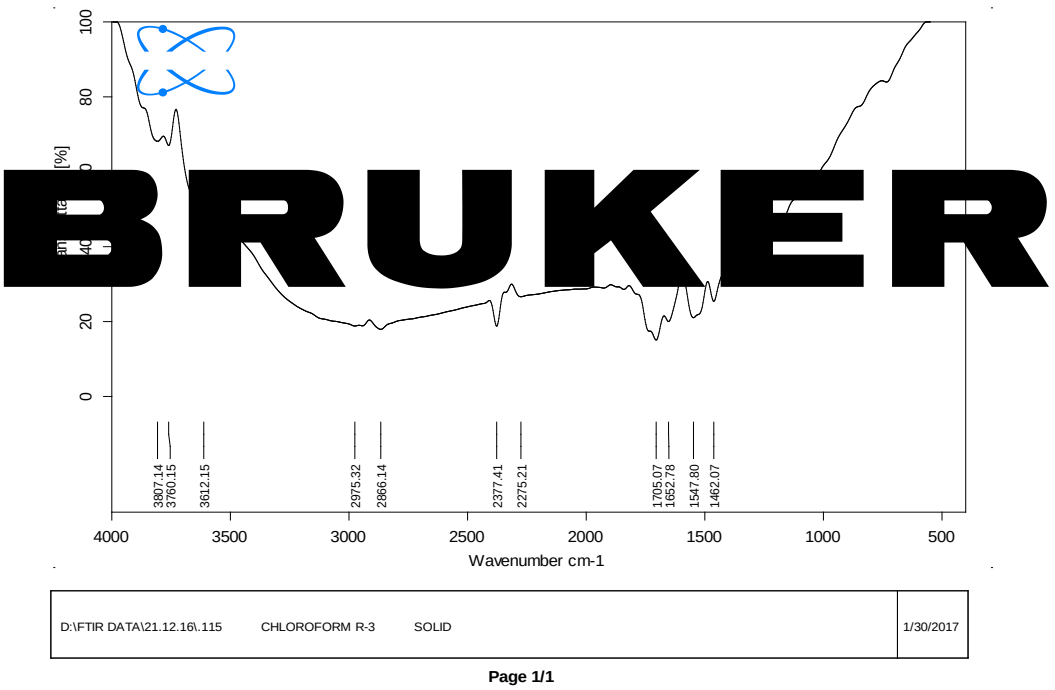

Fig. 3: FTIR spectrum of chloroform extract of *Grewia tilifolia* leaf

Table 5: Ethyl acetate extract FTIR interpretation of compounds

Extract	Wavenumber (cm^{-1})	Functional group	Phytocompounds identified
Ethyl Acetate	3391.83	O-H stretching	Poly Hydroxy Compound
	2923.19	Asymmetric stretching of–CH(CH_2) vibration	Lipids,proteins
	2855.84	Symmetric stretching of–CH_2(CH_2) vibration	Lipids,proteins
	1728.50	C=O stretching	Carbonyl Compound
	1454.88	C=C-C Aromatic ring stretching	Aromatic Compound
	1379.79	O-H bening,alcoholic group	Phenol or tertiary alcohol

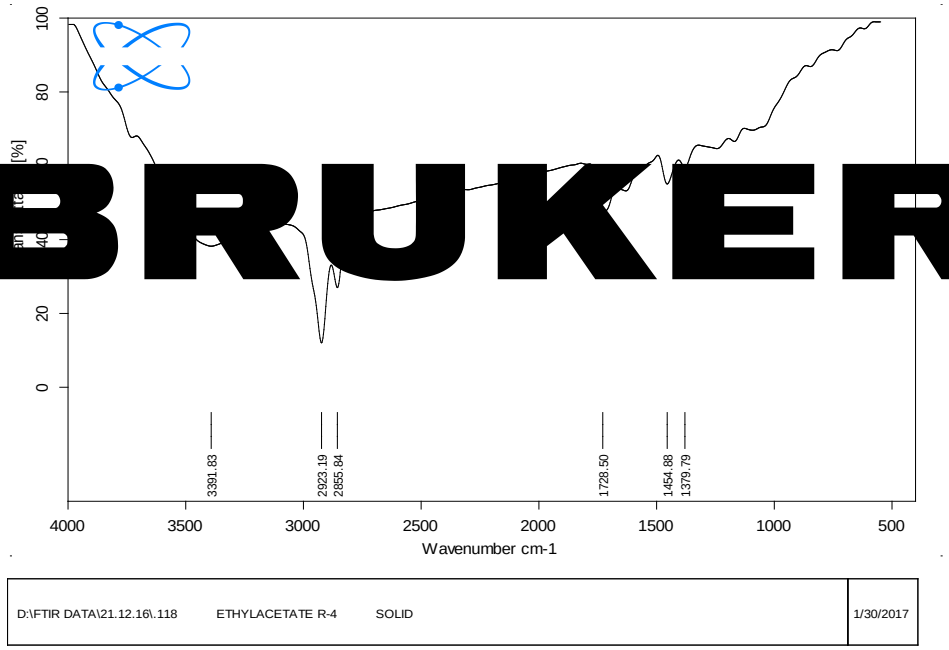

Fig. 4: FTIR spectrum of ethyl acetate extract of *Grewia tilifolia* leaf

Table 6: Acetone extract FTIR interpretation of compounds

Extract	Wavenumber (cm^{-1})	Functional group	Phytocompounds identified
Acetone	3424.53	O-H stretching,H-bonded	Hydroxy Compound
	2925.38	Symmetric stretching of–CH_2(CH_2) vibration	Lipids,proteins
	2856.55	Symmetric stretching of–CH_2(CH_2) vibration	Lipids,proteins
	1732.03	C=O stretching	aldehyde Compound
	1628.03	C=O stretching vibrations,ketone group	Ketone Compound
	1453.46	C=C-C Aromatic ring stretching	Aromatic Compound
	1380.08	O-H bening,alcoholic group	Phenol or tertiary alcohol
	1241.35	C-N stretching vibrations	Aromatic primary amine
	1169.18		
	1084.27	C-O stretching,Ether group	Cyclic ether
	838.69	P-O-C stretching	Aromatic Phosphate
	724.73	C-Cl stretching	Aliphatic Chloro Compound

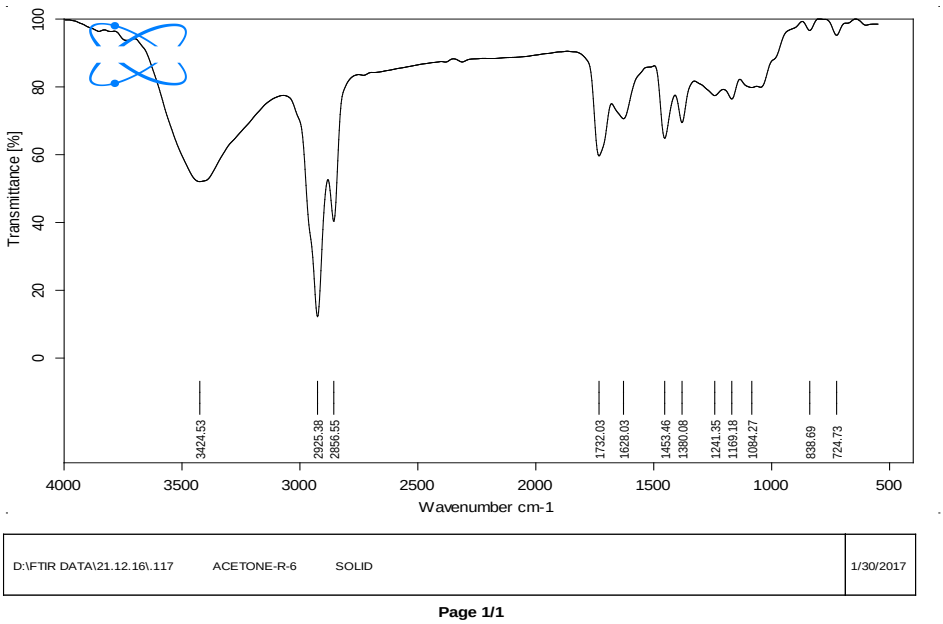

Fig. 5: FTIR spectrum of acetone extract of *Grewia tilifolia* leaf

Table 7: Benzene extract FTIR interpretation of compounds

Extract	Wavenumber (cm^{-1})	Functional groups	Phytocompounds identified
Benzene	3832.23	Non bonded,Ohstretching	Hydroxy group
	3742.13	Non bonded,Ohstretching	Hydroxy group
	3612.67	Non bonded,Ohstretching	Hydroxy group
	3565.07	O-H stretching,Hydroxy group (Intermolecular hydrogen-bonded OH)	Poly Hydroxy Compound
	2377.67		
	2303.30	C=C stretching(chelating compounds)	
	1834.16	Carbonyl Compound frequency	Transition metal carbonyls
	1742.02	C=O stretching	Ester
	1700.22	C=O stretching Carbonyl	Carbonyl Compound
	1652.52	C=O stretching	Amide
	1543.27	C=C stretching	Aromatic
	1521.22	C=C stretching	Aromatic
	1470.45	C=C-C Aromatic ring stretching	Aromatic Compound

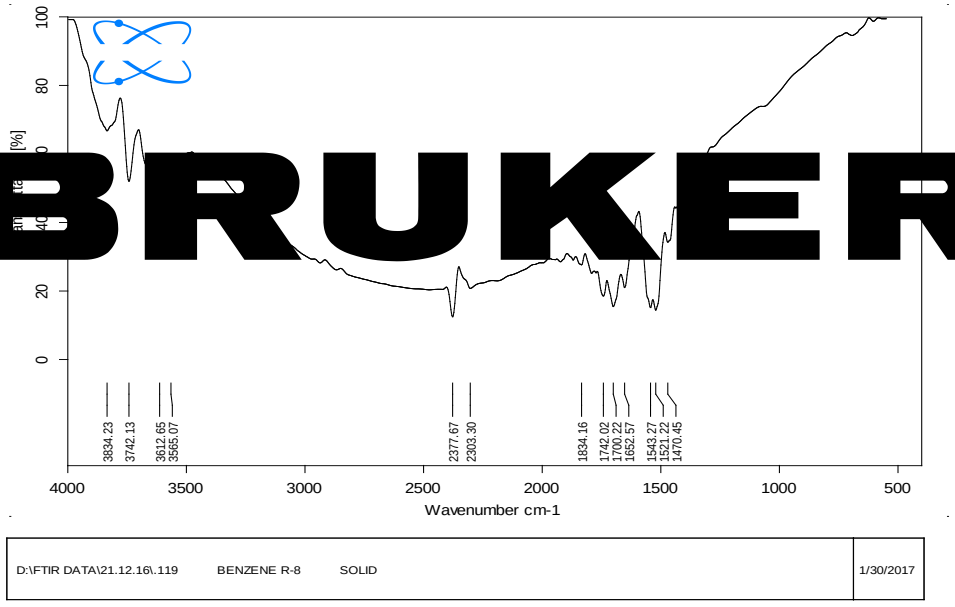

Fig. 6: FTIR spectrum of benzene extract of *Grewia tilifolia* leaf

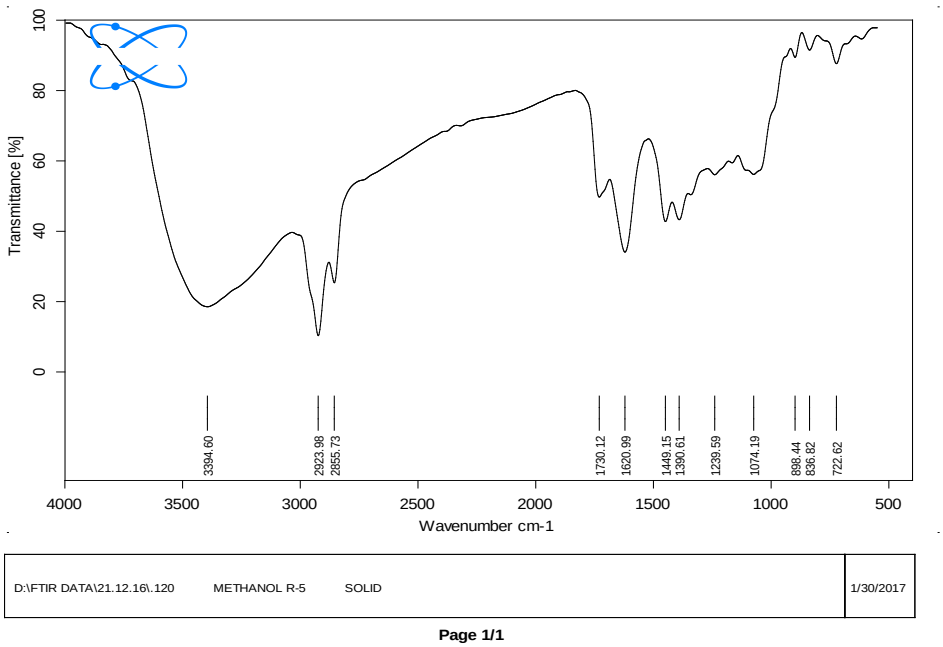

Fig. 7: FTIR spectrum of methanolic extract of *Grewia tilifolia* leaf

Table 8: Methanolil extract FTIR interpretation of compounds

Extract	Wavenumber (cm^{-1})	Functional Groups	Phytocompounds identified
	3394.60	O-H stretching H-bonded	Hydrogen Compounds
Methanol	2923.98	Asymmetric stretching of–CH(CH_2) vibration	Saturated aliphatic Compound-Lipids
	2855.73	Symmetric stretching of–CH_2(CH_2) vibration	Lipids,proteins
	1730.12	C=Ostretching	Aldehyde Compound
	1620.99	C=Ostretching	Ketone Compound
	1449.15	C=C-C Aromatic ring stretching	Aromatic Compound
	1390.61	O-H bending,Alcoholic group	Phenol or tertiary alcohol
	1239.59	CN stretching	Aromatic primary amine
	1074.19	Phasphate ion	Phasphate Compound
	898.44	P-O-C stretching	Aromatic phosphates
	836.82	P-O-C stretching	Aromatic phosphates
	722.64	C-Cl stretching	Aliphatic Chloro Compound

Table 9: Distilled water extract FTIR interpretation of compounds

Extract	Wavenumber (cm^{-1})	Functional groups	Phytocompounds identified
Aqueous	3416.31	N-H stretching	Amine
	1627.24	C=O stretchingvibration, ketone group	Ketone Compound
	1404.14	O-H bend Alcoholic group	Phenol or tertiary alcohol
	1338.15	CN stretching	Aromatic primary amine
	1275.13	CN stretching	Aromatic primary amine
	1121.28	C-O stretching, polymeric OH	Cycli ether
	936.74	P-O-C stretching	Aromatic phosphates
	897.36	P-O-C stretching	Aromatic phosphates
	719.91	C-Cl stretching	Aliphatic Chloro Compound

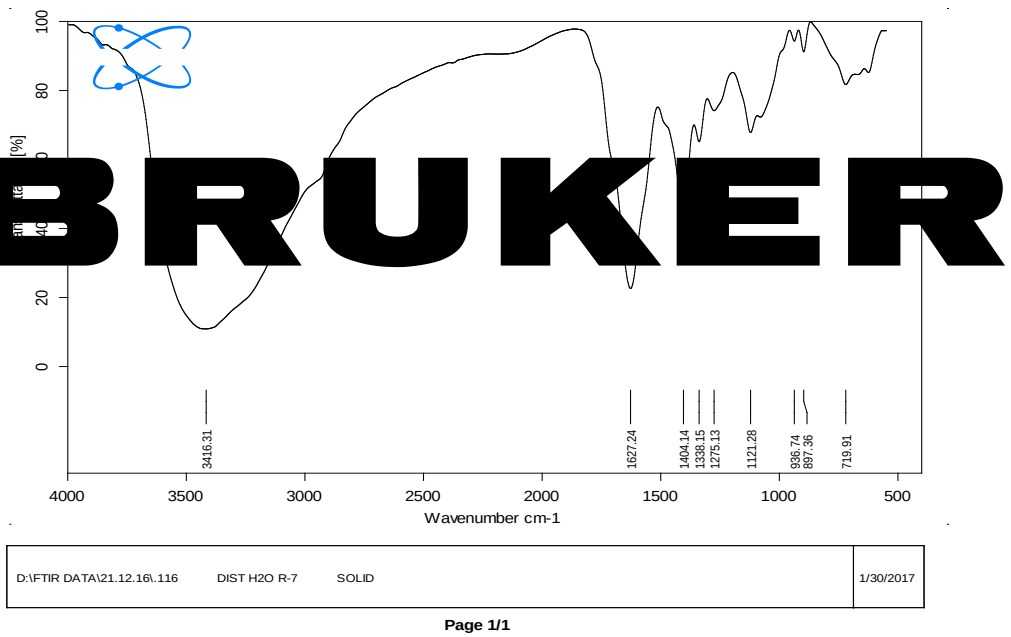

Fig. 8: FTIR spectrum of aqueous extract of *Grewia tilifolia* leaf

DISCUSSION

The pharmacological action of crude drugs and other therapeutic uses are due to their therapeutically active constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds or secondary metabolites of plants which serve as defense mechanism against predation by many microorganisms, insects and herbivores. So, the preliminary phytochemical analysis revealed pronounced importance because the crude drugs possess varied composition of secondary metabolites [27, 28]

The FTIR anaylsis revealved the prescence of alkaloids due to N-H stretching, polyphenols and flavonoids due to O-H stretching, terpens due to C-H group [29]. The functional groups present in test plant are aldehydes, alkenes, amines, amides, alcohols, phenols, aromatics, carboxylic acids and anhydride, esters and lactones, ethers and organic halogen compounds. These were confirmed by FT-IR spectrophotometer study that predicted the presence of the groups: O-H, N-H, C-H, CCl, C=C, nitrates and silicates stretching. The presence of characteristic functional groups of carboxylic acids, anhydrides, alcohols, phenols, amines, amides, esters, ethers, sulphur derivatives, glycosides, nitrates, nitriles, isonitriles, organic halogens and carbohydrate could be responsible for the various medicinal properties of *Grewia tilifolia* [29].

CONCLUSION

In the present study analysis of different extracts of *Grewia tilifolia* (vahl) leaf was done under FTIR will act as Pharmacognostic marker to distinguish the medicinally important Grewia species this

spectrocopic techinque is relatively simple,cost effective and can be usefull to easily detect functional groups. The results of present study is a way to predict and compare the phytoconstituents present in this plant with other bioactive medicinally important plants. Further the bioactive compounds need to be isolated and the structure of the compounds can be determined by using advanced analytical techniques such as Mass and NMR Spectrophotometers.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICTS OF INTERESTS

The authors have no conflict of interest

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