

Fourier Transform Infrared (FTIR) Spectroscopy of *Gmelina arborea* Roxb. Leaf Extracts

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Abstract

Gmelina arborea Roxb. is belong to the family of Verbenaceae, have been used for a long time in traditional medicine to treat a wide range of medical ailments. Through the use of the FTIR spectroscopy technique, the objective of this research is to investigate the effects of the extracts of *Gmelina arborea* leaves obtained from methanol, hydro alcohol, aqueous, hexane, and ethyl acetate. FTIR, which stands for "Fourier transform infrared spectroscopy," is an analytical method that is both rapid and nondestructive. In order to determine the usual peak values and the functional groups that are present in *Gmelina arborea* leaf extracts, Fourier transform infrared spectroscopy (FTIR) was used on a spectrophotometer system. FTIR testing confirmed that each extract did, in fact, contain a variety of functional groups that were biologically active.

Keywords: *Gmelina arborea* Roxb, Verbenaceae, FTIR, Spectroscopy, Functional Groups

Introduction

India is the country that produces the most useful plants in the world, thus it is only fitting that it is referred to as the botanical paradise (Mewada et al., 2021). It has a broad variety of over 45,000 plant species, 7000 of which are considered to be medicinal herbs, and it is one of the 12 main biodiversity hotspots in the world. There are 16 agro-climatic zones in this region (Prasathkumar et al., 2021). About twenty percent of all plants that are now known have been used in pharmacological research. This has resulted in significant advancements in the medical field, particularly in the treatment of cancer and other diseases. Since ancient times, many ailments have been treated using plant-based remedies that are considered to be part of folk medicine (Omodamiro et al., 2020).

The plant known as *Gmelina arborea* Roxb. belongs to the family Verbenaceae. It may be found in a significant amount of India, including the Western Ghats, the foothills of the North-West Himalaya, Chittagong, and the Deccan Peninsula, among other locations. It is a large to medium-sized deciduous tree that may reach a height of between 15 and 20 meters (Figure: 1). (Pathala et al., 2015). *G. arborea* is one of the medicinal plants whose curative properties have been established in a number of studies for a wide range of human ailments. These studies were conducted using a variety of research methods. The root, the leaf, and the bark of the *Gmelina arborea* all contain anti-inflammatory activities (Merlin et al., 2009), antibacterial qualities (El-Mahmood et al., 2010; Audipudi & Chakicherla, 2010), and antioxidant capabilities (Merlin et al., 2010). (Audipu I & Chakicherla, 2010). A phytochemical screening indicated the presence of polysaccharides, saponins, tannins,

anthraquinones, and cardiac glycosides. In addition, it has flavonoid, alkaloid, glycoside, lignan derivatives, and sesquiterpenoid (Chothani & Patel, 2012; Soni & Sosa, 2013).

Spectroscopy has recently emerged as one of the most essential tools for use in biomedical applications, and as a result, significant advancements have been made in the area of clinical evaluation. Spectroscopic techniques, such as FTIR spectroscopy, have been used to the investigation of a wide range of natural tissues. These vibrational spectroscopy methods are simple, easy to replicate, and harmless to the tissue. In addition, they call for only very small amounts of material (ranging from micrograms to nanograms) and need just the barest minimum of sample preparation. In addition, these methods provide information at the molecular level that may be put to use in the investigation of functional groups, different forms of bonding, and various conformations of molecules (Baseri & Baker, S2011). It is already common knowledge that FTIR spectroscopy may be used to provide an accurate description of biological tissues. FTIR spectroscopy is quickly becoming the method of choice for scientists researching chemical structural characteristics in natural as well as synthetic tissues (Bunaciu et al., 2010). Analysis of functional groups is very important for gaining a comprehensive understanding of the physicochemical properties of the extract. Determining the functional group also helps in assessing the structure-activity correlations of the compound (Poojary et al., 2015).

The objective of this study was to conduct an investigation into the functional group analysis of *Gmelina arborea* leaf extracts. Extraction methods such as the Soxhlet methanolic extract (GMM1), the hydroalcoholic extract (GMHA), and the aqueous extract were all used (GMA). Hexane extract (GMHE), ethyl acetate extract (GMEA), and methanolic extract were all obtained using the exhaustive serial extraction procedure (GMM2).



Figure 1. *Gmelina arborea* Roxb., A: Leaves; B: Flowers; C: Fruits

Methods

Chemicals

The majority of the chemicals were purchased from Hi-Media in Mumbai, India.

Plant Collection

Gmelina arborea leaves were gathered in December 2021 from Ahmedabad district, Gujarat, India (Latitude 23.01° N, Longitude 72.59° E); it was quickly recognized and pre-treated. With the reference identification GU/BOT/V/G06/2022, the plant leaves were authenticated by the Department of Botany at Gujarat University.

Leaf Extracts Preparation

Extracts Preparation Using Soxhlet & Maceration Method

It is acceptable to eliminate the fungal and parasitic infections that have taken hold on the affected leaves. After being adequately cleaned once with tap water, the leaves of *G. arborea*

were washed a total of three times with distilled water in order to remove any dust and other particles (DW). Following a drying period of one month, the leaves were put through a mechanical grinder in order to reach the desired powder consistency. After that, the samples were kept at a temperature of 4 degrees Celsius for further examination. For the production of a methanolic extract, fifty grams of *G. arborea* leaf powder was encased in a Soxhlet apparatus together with four hundred and fifty milliliters of 99% methanol (GMM1). The extraction was carried out at 65 degrees Celsius for sixteen hours, with two to three cycles carried out each hour, until the extractive became colorless. The maceration process was also utilized to make a 10% hydroalcoholic extract (GMHA; DW: Methanol; 1:1v/v), in addition to a 10% aqueous extract. Both of these extracts had a ratio of 1 part water to 1 part methanol (GMA). Following the completion of the extraction process, all of the materials were filtered using Whatman No. 1 filter paper and accumulated in a Petri dish. The filtrates were dried in a hot air oven for 20 hours at a temperature of 45 degrees Celsius (Electroquip). Each extract's yield was collected in a container made of amber and kept at a temperature of 4 degrees Celsius in order to prevent the bioactive component from being contaminated or degraded as a result of light or temperature. At last, the formula was used in order to determine the yield percentage of each crud extract (Table:1).

$$Y(\%) = \text{Final weight of extract obtained} / \text{Initial weight of raw material} \times 10$$

Preparation of Extraction Samples Using Serial Exhaustive Extraction Method

Extractions of *Gmelina arborea* leaves were made using a technique called the Serial Exhaustive Extraction Method. 1g of G. The powder of arborea was placed in beakers with a capacity of 50 milliliters, and then it was macerated twice with (hexane, ethyl acetate, and methanol) in a water bath at a temperature of 30 degrees Celsius for ten minutes. After that, it was filtered using Whatman No.1 filter paper. Following each stage of extraction, the residues were put through a progressive extraction using solvents of progressively increasing polarity. To get 10% ethyl acetate and 10% methanol, the residue from the hexane extraction was sequentially extracted with ethyl acetate and methanol (GMHE, GMEA, and GMM2). The organic filtrates were dried in a hot air oven by Electroquip at a temperature of 45 degrees for a period of 14 hours. until a crude extract was obtained, after which the extracts were stored in sterile MCT at a temperature of 4 degrees Celsius for use in further study.

Fourier-Transform Infrared Spectroscopy Analysis (FTIR)

On a number of different sample formats, including GMM1, GMHA, GMA (solid powder extract), and GMHE, GMEA, and GMM2, functional group analysis was carried out (liquid extract). Investigations were conducted on frequencies ranging from 500 cm⁻¹ to 3500 cm⁻¹ as part of the documentation for functional groups. For the purpose of recording all of the measurements in transmittance mode, a Bruker Alpha from Lab India Instrument Private Limited was employed. This instrument was controlled by OPUS 7.5 software.

Results and Discussion

Yield Value

The current research emphasizes the importance of yield in determining the active elements of *Gmelina arborea* leaves as a medicinal raw material. According to the results, the GMHA extract generated the highest percentage yield, followed by the GMA and GMM1 (Table:1).

Table 1. Yield of various extracts of *Gmelina arborea*

Extraction method	Extraction solvent	% Yield
Soxhlation	Methanol (GMM1)	4.72%

Maceration	Hydro-alcoholic (GMHA)	14%
Maceration	D.W (GMA)	7.9%

Fourier-Transform Infrared Spectroscopy Analysis (FTIR)

G. arborea extracts were analyzed using an FTIR spectrum to identify functional groups and peaks typical of infrared radiation. Between 1000-4000 cm^{-1} (12, 13, 9, 18, 21, 13) noteworthy bands were found in GMM1, GMHA, GMA, GMHE, GMEA, and GMM2 respectively (Table: 2,3; Figures: (2, 3, 4, 5, 6, 7, 8)). The Maximum band appeared with GMEA twenty-one bands with different functional groups (Figure: 6). The bending vibrations of alcohol/phenol (O-H stretch) were detected in GMEA spectra at (3844.1 cm^{-1} , 3743 cm^{-1} , 3677.6 cm^{-1} , 3647.6 cm^{-1} , and 3619 cm^{-1}). Further peaks, (2984.3 cm^{-1} , 2918.4 cm^{-1}) show strong N-H stretching, indicating that the amine salt is present. At 2850.8 cm^{-1} vibrations, a (C-H bending medium) was recorded. Carbon dioxide was detected at 2362.8 cm^{-1} with (O=C=O stretching strong). three bands were identified between 1500-2000 cm^{-1} , 1736.6 cm^{-1} (C=O stretching strong) 1648.72 cm^{-1} (C-C stretching medium) and 1515.26 cm^{-1} (N-O stretching medium). Five bands appeared in the frequency range of 1000-1500 cm^{-1} amongst two peaks indicating bands of C-H bending (1458.52 cm^{-1} , 13714 cm^{-1}) and two peaked (1232.3 cm^{-1} , 1098.8 cm^{-1}) assigning C-H stretching bands and 1042.9 cm^{-1} assigning C-O stretching bands. Between the rang 1000-500 cm^{-1} displayed bands at 934.7 cm^{-1} and 786.3 cm^{-1} (C=C bending strong), 846.9 cm^{-1} (C-Cl stretching strong) at last 633.6 cm^{-1} (C-Br stretching strong).

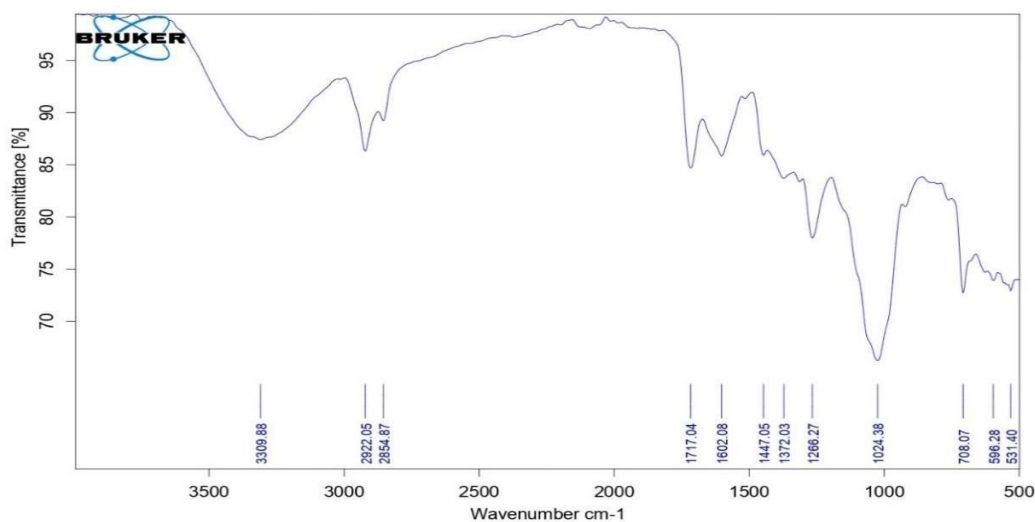


Figure 2. FTIR spectrum of GMM1 extract

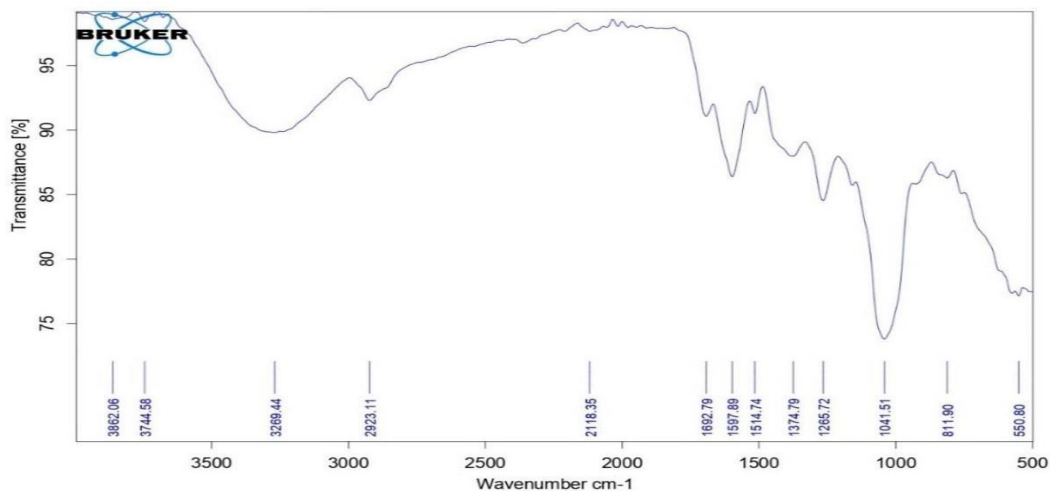


Figure 3. FTIR spectrum of GMHA extract

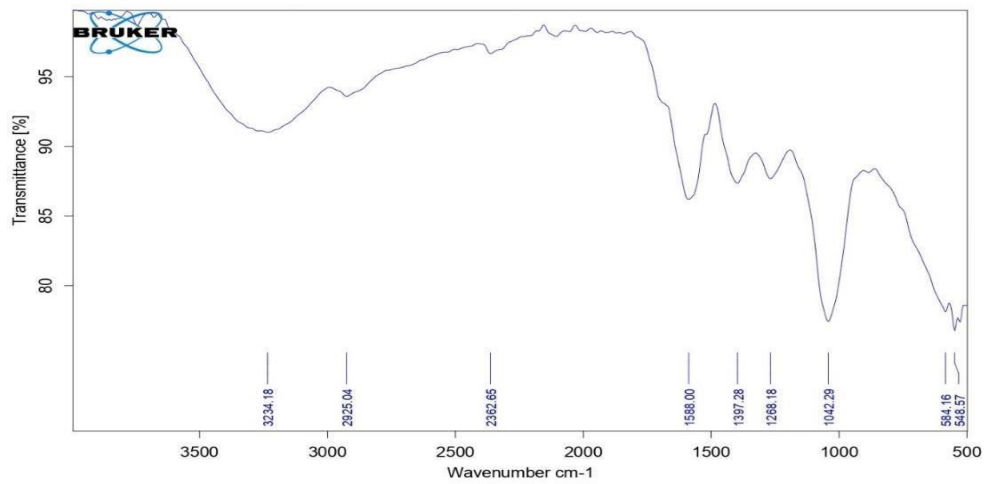


Figure 4. FTIR spectrum of GMA extract

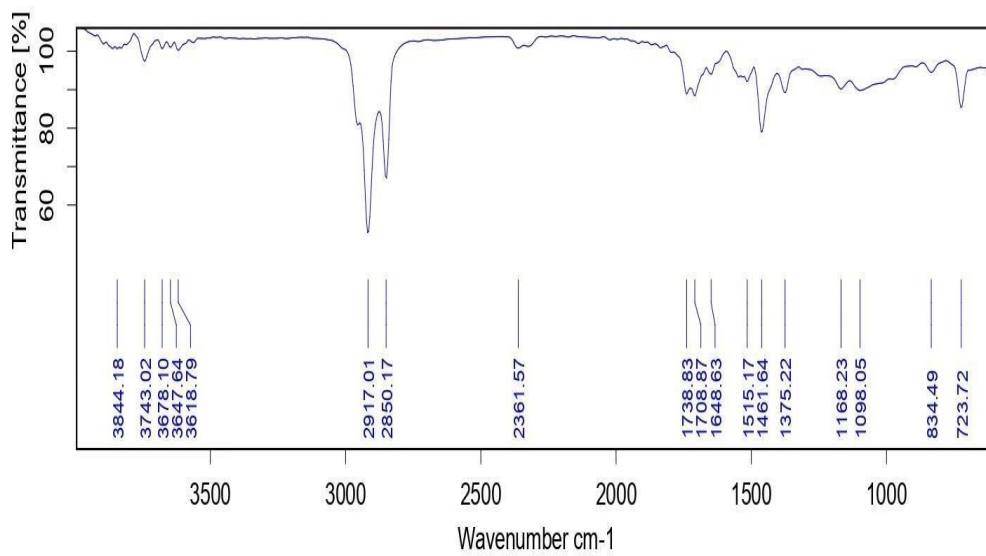


Figure 5. FTIR spectrum of GMHE extract

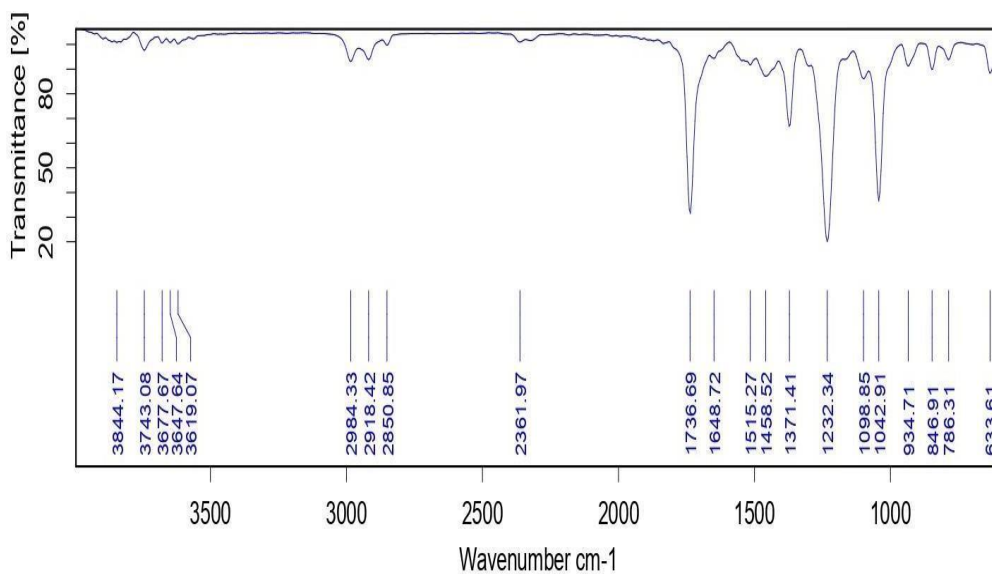


Figure 6. FTIR spectrum of GMEA extract

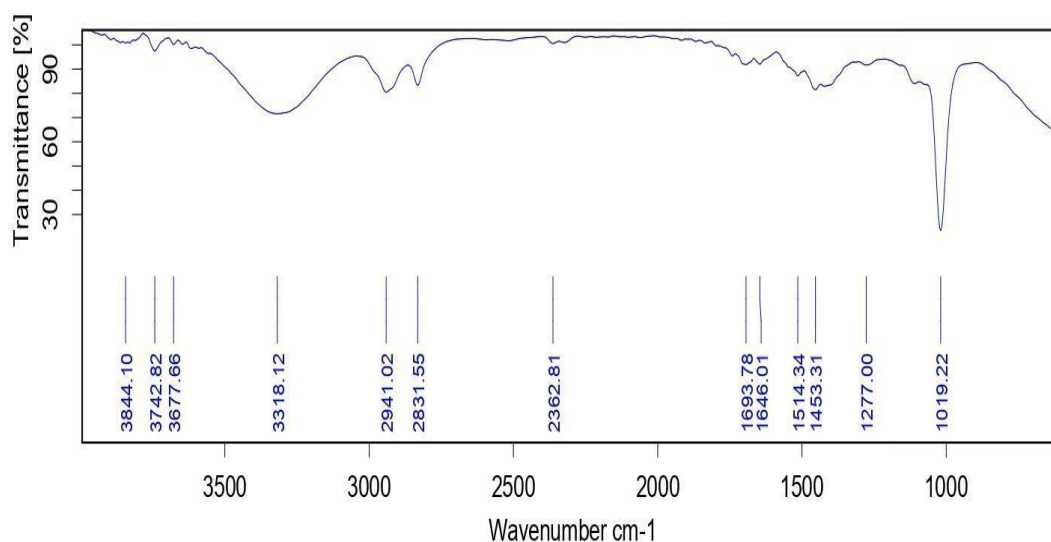


Figure 7. FTIR spectrum of GMM2 extract

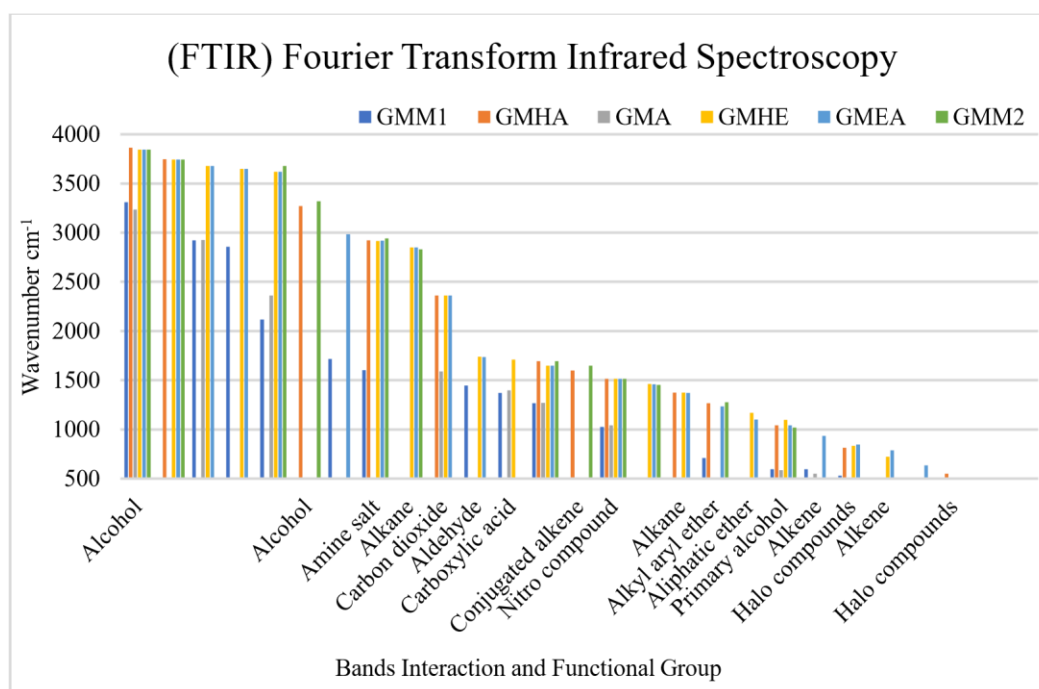


Figure 8. Functional groups in various extracts of *Gmelina arborea* leaves

Table 2. FTIR analysis revealed the presence of functional groups of GMM1, GMHA, and GMA extracts of *Gmelina arborea*

Peak Rang cm-1	Peak Value			Appearance	Bands Interaction and Functional Groups	Possible Compounds
	GMM1	GMHA	GMA			
4000-3500		3862.06		Strong, Broad	O-H stretching	Alcohol
		3744.58		Strong, Broad	O-H stretching	Alcohol
3500-3000	3309.88	3269.44	3234.18	Strong, Broad	O-H stretching	Alcohol
3000-2500	2922.05	2923.11	2925.04	Strong, Broad	N-H stretching	Amine Salt
	2854.87			Medium	C-H stretching	Alkane

2500-2000		2118.35	2362.65	Strong	O=C=O stretching	Carbon Dioxide
2000-1500	1717.04			Strong	C=O stretching	Carboxylic Acid
	1602.08	1692.79		Medium	C-C stretching	Conjugated Alkene
		1597.89		Medium	C-C stretching	Conjugated Alkene
		1514.74	1588	Medium	N-O stretching	Nitro Compound
1500-1000	1447.05			Medium	C-H bending	Alkane
	1372.03	1374.79	1397.28	Medium	C-H bending	Alkane
	1266.27	1265.72	1268.18	Strong	C-H stretching	Alkyl Aryl Ether
	1024.38	1041.51	1042.29	Strong	C-O stretching	Primary Alcohol
1000-500		811.9		Strong	C-Cl stretching	Halo Compound
	708.07			Strong	C=C bending	Alkene
	596.28	550.8	584.16	Out of ring	C-halogen	Halo Compounds
			548.57			
	531.4					

Table 3. FTIR analysis revealed the presence of functional groups of GMHE, GMEA, and GMM2 extracts of *Gmelina arborea*

Peak Rang cm-1	Peak Value			Appearance	Bands Interaction and Functional Groups	Possible Compounds
	GMHE	GMEA	GMM2			
4000-3500	3844.18	3844.1	3844.1	Strong, Broad	O-H stretching	Alcohol
	3743.02	3743	3742.82	Strong, Broad	O-H stretching	Alcohol
	3678.1	3677.6		Strong, Broad	O-H stretching	Alcohol
	3647.6	3647.6		Strong, Broad	O-H stretching	Alcohol
	3618.79	3619	3677.66	Strong, Broad	O-H stretching	Alcohol
3500-3000			3318.12	Strong, Broad	O-H stretching	Alcohol
3000-2500		2984.3		Strong, Broad	N-H stretching	Amine Salt
	2917.01	2918.4	2941.02	Strong, Broad	N-H stretching	Amine Salt
	2850.17	2850.8	2831.55	Medium	C-H stretching	Alkane
2500-2000	2361.9	2361.9	2362.81	Strong	O=C=O stretching	Carbon Dioxide
2000-1500	1738.8	1736.6		Strong	C=O stretching	Aldehyde
	1708.87			Strong	C=O stretching	Carboxylic Acid
	1648.64	1648.72	1693.78	Medium	C-C stretching	Conjugated Alkene
			1646	Medium	C-C stretching	Conjugated Alkene

	1515.17	1515.27	1514.34	Medium	N-O stretching	Nitro Compound
1500-1000	1461.64	1458.52	1453.31	Medium	C-H bending	Alkane
	1375.22	1371.4		Medium	C-H bending	Alkane
		1232.3	1277	Strong	C-H stretching	Alkyl Aryl Ether
	1168.23	1098.8		Strong	C-H stretching	Aliphatic Ether
	1098.05	1042.9	1019.22	Strong	C-O stretching	Primary Alcohol
1000-500		934.7		Strong	C=C bending	Alkene
	834.49	846.9		Strong	C-Cl stretching	Halo Compound
	723.72	786.3		Strong	C=C bending	Alkene
		633.6		Strong	C-Br stretching	Halo Compounds

The understanding the overall physicochemical parameters of the extract depends heavily on functional group analysis. In addition, determining the functional group aids in evaluating the structure-activity correlations (Poojary, 2015). The presence of hydrogen-bonded OH group phytochemicals was confirmed by FTIR spectroscopy of all the extracts. Most phenolic phytochemicals, including flavonoids, have hydroxyl activity. Furthermore, our results suggest that *G. arborea* leaf extracts include a variety of physiologically active functional groups, including amines, amides, alcohols/phenols O-H stretch, carboxylic acids, aldehydes, etc. (Table: 2, 3). As a result, we can prove that the plant contains significant bioactive phytochemicals.

Conclusion

In conclusion, the results indicate that the leaves of *G. arborea* might be a source of phytoconstituents. This conclusion was reached based on the findings of the study. The current investigation is supposed to find particular molecules that may be utilized to examine new and more effective bioactive manufactured from plants. This is something that is predicted to be discovered. In order to separate and identify active molecules in the crude extract, further study is required. Additionally, it is necessary to conduct an exhaustive investigation into the in silico, in vitro, and in vivo biological activity of the recovered compounds.

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