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FTIR Analysis and Phytoconstituents Screening of *Aegle Marmelos* L. Leaves in Various Extracts

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ABSTRACT: Medicinal plants are used in traditional medicine to cure various ailments. They consist of a number of bioactive components like alkaloids, flavonoids, glycosides, steroids, tannins, phenolic compounds, etc. are used for the treatment of several diseases. *Aegle marmelos* L. is a spiritual, religious and important medicinal plant commonly called bael. Different parts of bael used in ethnomedicine are known to possess antioxidant, antidiarrheal, antibacterial, antidysenteric, anti-inflammatory, antipyretic, analgesic activities. The aim of the present investigation is to carry screening of phytochemical constituents and Fourier Transform Infrared spectroscopic analysis of various extracts of *Aegle marmelos* L. leaves. The preliminary screening of phytoconstituents was carried out by standard methods. In the *Aegle marmelos* L. leaves the presence of phytochemicals such as carbohydrates, proteins, amino acids, steroids, alkaloids, flavonoids, glycosides, saponins, tannins were present in aqueous, acetone, ethyl acetate, dichloromethane, ethanol, methanol and petroleum ether extract. Results of the FTIR spectra of leaves extracts revealed the presence of various functional groups like alkanes, aldehydes, amides, alcohols, esters, ethers, carboxylic acids, carbonyl group, aromatic compounds, etc. All these compounds pertain to the bioactive components and could be responsible for the medicinal properties of *Aegle mermelos* L.

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INTRODUCTION

Plants are natural resources of bioactive constituents used in the treatment of various disorders. The medicinal plants and their derivatives have been perceived as an essential source of therapeutically effectual medicine as they contain secondary metabolites, which are potential sources of drugs [1]. The most important bioactive constituents present in medicinal plants are flavonoids, carboxylic acids, carbohydrate, phenols, tannins, alkaloids, terpenoids, steroids and amino acids, etc. [2]. The phytochemical constituents playing a crucial role in medicines can be identified using crude extracts of the plants [3]. Plant crude extracts were containing a high amount of natural antioxidants, which are used as folkloric medicines [4]. Since ancient times, plant extracts were used in medical practices for the treatment of various ailments [5].

Aegle marmelos L. popularly known as Bael in India belonging to the family Rutaceae is an important aromatic medicinal plant that has enormous traditional uses against various diseases and many bioactive compounds have been isolated from this plant also. It is widely grown in India, tropical and subtropical countries [6].

Various parts of the *Aegle marmelos* L.viz. roots, leaves, stem, bark and seeds are reported to have medicinal value [7,8]. These parts are used for the treatment of a myriad ailments such as chronic diarrhoea, dysentery, peptic ulcer, inflammation and diabetes is documented [9, 10, 11, 12]. Different parts of the plants contain hypoglycaemic, hypolipidemic and blood pressure- lowering factors [13].

Several investigations reported that *Aegle marmelos* L. has been possessing important pharmacological properties including antioxidant, anticancer, anti-inflammatory, antipyretic, analgesic, anti-hyperglycemic and antiproliferative

effects [14, 15, 16, 17, 18]. A number of scientific studies validated that *Aegle marmelos* L. contains numerous phytochemicals in high levels, such as carotenoids, phenolic, alkaloids, pectins, tannins, coumarins, flavonoids and terpenoids [8, 19, 20, 21]. Sawale *et al* (2018) reported that the number of phytoconstituents are present in bael makes useful for several ailments [21]. The leaves are used as febrifuge, astringent, laxative, digestive and expectorant. These are useful in the treatment of abdominal pain, fever, asthma, heart palpitation, stomach pain, ophthalmia, urinary troubles, ulcers, vomiting and swellings [22, 23, 24, 25, 26]. The leaf extracts of bael is used as a medication against a number of chronic diseases like diabetes, pancreatic cancer and arthritis [27, 11, 28, 29]. Different organic extracts of the leaves of *Bael (A. marmelos)* have been reported to possess alkaloids, cardiac glycosides, terpenoids, saponins, tannins, flavonoids and steroids [30, 31, 32].

Various techniques can be used to determine and estimate the presence of such bioactive components in medicinal plants. The Fourier Transform Infrared (FTIR) spectroscopic technique is a high-resolution analytical technique to identify the chemical constituents and elucidate the structural compounds [33, 34]. In recent years, FTIR has played a significant role in pharmaceutical analysis for the fingerprint characters and extensive applicability to the samples. Moreover, the FTIR spectroscopy is a rapid, time saving and valuable tool for the characterization and identification of the functional groups present in the plant extracts or powders. Therefore, the aim of the present study was to find out the screening of phytochemical constituents and FTIR analysis in various solvent extracts of *Aegle marmelos* L. leaves.

MATERIAL AND METHODS

Collection and identification of Plant material

The fresh leaves of *Aegle marmelos* L. were collected by taking all the precautions and by avoiding damage to the plant life. The collected plant materials were identified and taxonomically authenticated (TGD-1) by the Scientist D and HOD, Botanical Survey of India, Pune, Maharashtra.

Preparation of plant extracts

The fresh leaves were collected and washed under tap water to remove adhering dust and then dried in the shade at ambient temperature. The shade dried leaves were powdered in a mechanical grinder. The coarse leaf powder was subjected to the successive extraction with different solvents viz. water, acetone, ethyl acetate, dichloromethane, ethanol, methanol and petroleum ether using maceration technique. Then the crude extracts were filtered using Whatman No.1 filter paper. The supernatant was collected, evaporated and concentrated into solid extracts under room temperature. It was stored at $4^{O}C$ in airtight bottles for subsequent analysis.

Phytochemical Screening

Preliminary phytochemical analysis was carried out identification of various secondary metabolites present in aqueous, acetone, ethyl acetate, dichloromethane, ethanol, methanol and petroleum ether by using standard methods [35, 36].

Fourier Transform Infrared Spectrophotometer (FTIR) Analysis

The dried leaves powder of different solvent extracts of *Aegle marmelos* L. were analyzed using 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 extract was loaded in FTIR spectrophotometer (Shimadzu, Japan), with a scan range from 400 to 4000 cm-1 with a resolution of 4 cm-1 and FTIR spectrum was recorded. The FTIR spectra were used to determine the presence of functional groups in the leaf extracts of *Aegle marmelos* L.

RESULTS AND DISCUSSION

Phytochemical Analysis

The results of phytochemical analysis of the aqueous, acetone, ethyl acetate, dichloromethane, ethanol, methanol and petroleum ether extract of *Aegle marmelos* L. leaves are shown in **Table 1.** It revealed the presence of active constituents like alkaloids, flavonoids, amino acids, steroids, saponins, tannins, glycosides, anthraquinone glycosides, carbohydrates, proteins and phenolic compounds. However, alkaloids were present only in aqueous and ethanolic extracts and the cardiac glycosides were found in ethyl acetate and acetone extracts of *Aegle marmelos* L. leaves. Next to aqueous, methanol and ethanol extracts were found to be more significant as compared to ethyl acetate, acetone and petroleum ether extracts but dichloromethane extract showed less variety of phytochemical constituents. Tannins were observed in all extracts except petroleum ether extract of *Aegle marmelos* L. leaves.

In the present study, bioactive constituents like alkaloids, phenols, flavonoids, steroids and saponins were revealed in *Aegle marmelos* L. leaves extracts. This shows a high level of its possible medicinal values [37, 38, 39]. The phytochemical compounds such as phenols, sterols, tannins, flavonoids, saponins, coumarins and triterpenoids were present in extracts of leaves of *Aegle marmelos* L. [40]. Screening of plants for medicinal value has been carried out by several workers with the help of phytochemical analysis [41, 42, 43]. The presence of flavonoids, saponins, alkaloids, steroids, tannins and phenolic compounds possesses analgesic, anti-allergic, antioxidant, anticancer, antimicrobial, anticarcinogenic, anti-inflammatory and antibacterial activities [44, 45, 46, 47, 48, 49]. The present study clearly indicates that the presence of medicinally important phytochemicals may have accounted for the various therapeutic uses of the *Aegle marmelos* L. leaves.

Table 1: Phytochemical Screening of *Aegle marmelos* L. Leaves Extracts

Secondary Metabolites	Phytochemical tests	Nature of extract						
		Aqueous	Acetone	Ethyl acetate	Dichloro methane	Methanol	Ethanol	Petroleum ether
Carbohydrates	Molisch's Test	+	+	+	_	+	+	+
Protein	Millon's Reagent Test	+	_	+	_	+	+	_
Amino acids	Ninhydrin Test	+	+	+	_	+	+	_
Steroid	Liebermann Burchard Reaction	+	+	+	_	+	+	+
Glycosides a.Cardiac glycosides b.Anthraquinone glycosides c.Saponin glycosides	Legal's Test Borntrager's Test FoamTest	_ + +	+ _ +	+ _ +	_ _ +	_ + +	_ + +	_ _ _
Flavonoids	Sodium hydroxide Test	+	+	+	_	+	+	+
Phenols	Ferric Chloride Test	+	_	+	+	+	+	+
Alkaloids	Mayer's Test Wagner's Test	+ +	_ _	_ _	_ _	_ _	+ +	_ _
Tannins	Dilute Nitric acid Test	+	+	+	+	+	+	_

(+) = Positive (present) ; (-) = Negative (absent)

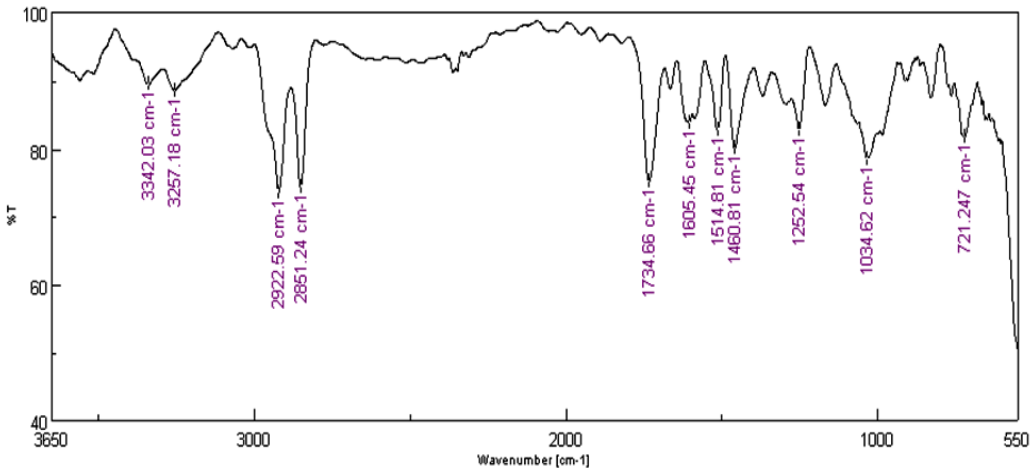

Figure 1: FTIR spectrum of Aqueous extract of *Aegle marmelos* L.

Table 2 : FTIR spectral wave numbers (cm-1) of dominant peak and functional groups obtained from the leaves extracts of *Aegle marmelos* L.

Functional group	Component (Peaks)	Wave numbers (cm-1)						
		Aqueous Extract	Acetone Extract	Ethyl Acetate Extract	Dichloromethane Extract	Ethanol Extract	Methanol Extract	Pet -ether Extract
Alkanes	C-H (stretch)	2851.2 2922.5	2852.2 2919.7	2852.2, 2923.6	-	2924.5	2923.5	2852.2 2921.6
	-CH_2 (bend)	-	1456	1458.8	-	1459.8	1460.8	1460.8
Alkenes	C-H(stretch)	-	-	3079	-	2851.2	-	-
	C=C(stretch)	1605.4	1607.38	1255.43	1590	1613.1	-	-
	C=C (bend)	721.2	-	832.1,720.2, 980.62	831.1	719.3	921.8	-
Aromatic rings	=C-H (bend)	-	-	720.28, 832.1	-	-	852.3	-
	=C-H(stretch)	721.2	-	-	-	-	-	-
	C=C (bend)	1514.8 1605.4	-	1613.1 1511.9	-	1510.9	1612.2	1589.06
Aldehydes	C=O(stretch)	1734.6	1730.8	1729.8	-	1734.6	1725.9	1735.6
Carboxylic acids and their salts	C=O (stretch)	1734.6	1730.8	1729.8	-		1725.9	
	C- O (stretch)	1460.8	1052.9	1458.8	-	1459.8	-	
Esters	C=O (stretch)	-	1730.8	1729.8	-	-	1725.9	1735.6
	C- O (stretch)	1252.5	1052.9	1255.4		1255.4	-	-
Primary Amines	N-H (stretch)	2922.5,3257.1 3342	2919.7	2923.6,3261.5	3393.1	3355.5	2923.06	2852.2,2921.6 3256.2,3336.2
	C-N (stretch)	-	1052.9	-	-	1032.6	1043.3	1021.
	N-H (stretch)	-	3570.5	-	3604.3	3544.5	3556.09	-
Ethers	C-O (stretch)	1034.6 1252.5	-	1255.4	1273.7	1032.6 1255.4	1043.3	1021.12
Alcohols	O-H (stretch)	2922.5 3342	2919.7 3362.2	2923.6, 3541 3079,3261	3393.14 3604.3	3355.5 3544.5	3211.8 3556.09	2921.6, 3256.2 3336.25
Ketone	C=O (stretch)	-	1730.8	-	-	-	1725.9	-
	C=C (stretch)	-	-	1613.1	-	1613.1	1612.2	-
Nitro compounds	N=O (stretch)	1514.8	-	1511.9	-	1510.9	-	-
Ammonium salts	C=O (stretch)	1460.8 1734.6	1730.8	1458.8	-	-	1460.8	-
Sulphonate salts	S=O (stretch)	-	-	1168.6	-	-	-	-
Carbonate ion	-	-	1456.9	1458.8	1424.17	1459.8	852.3,1460	1460.8
Sulfoxides	S=O (stretch)	-	1052.9	-	-	1032.6	1043.3	-
Halides	C-F C-Cl	-	-	1168.6	-	-	- 852.38	-

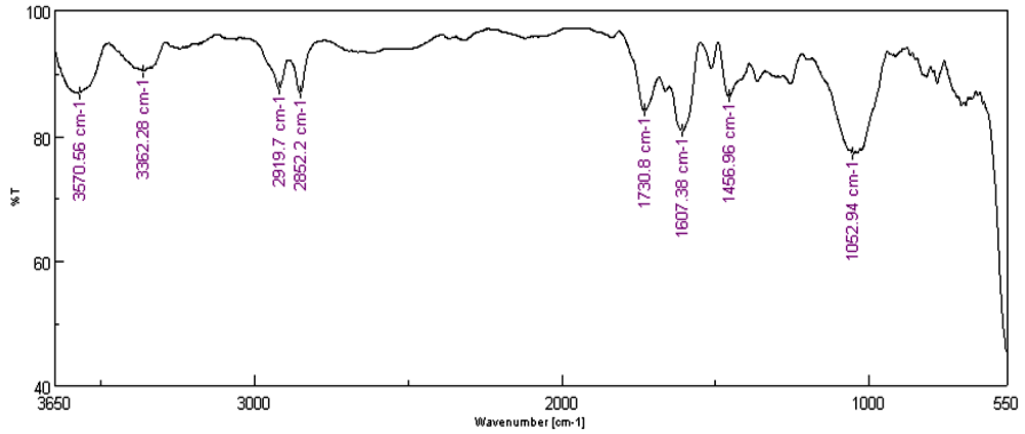

Figure 2: FTIR spectrum of Acetone extract of *Aegle marmelos* L.

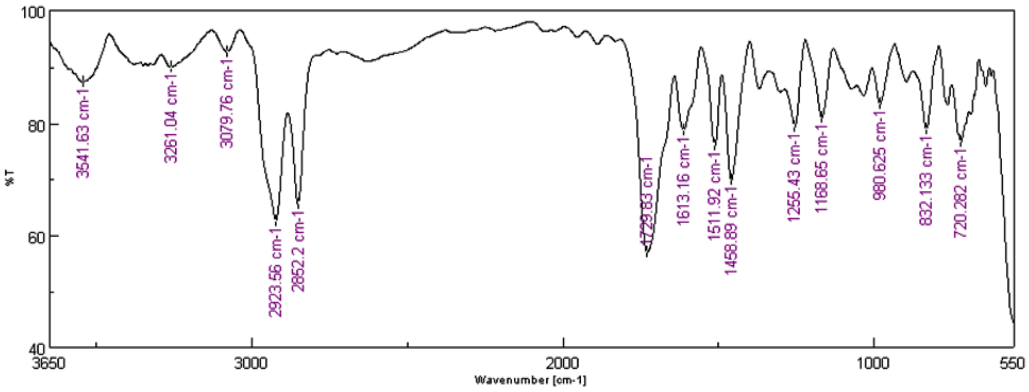

Figure 3: FTIR spectrum of Ethyl acetate extract of *Aegle marmelos* L.

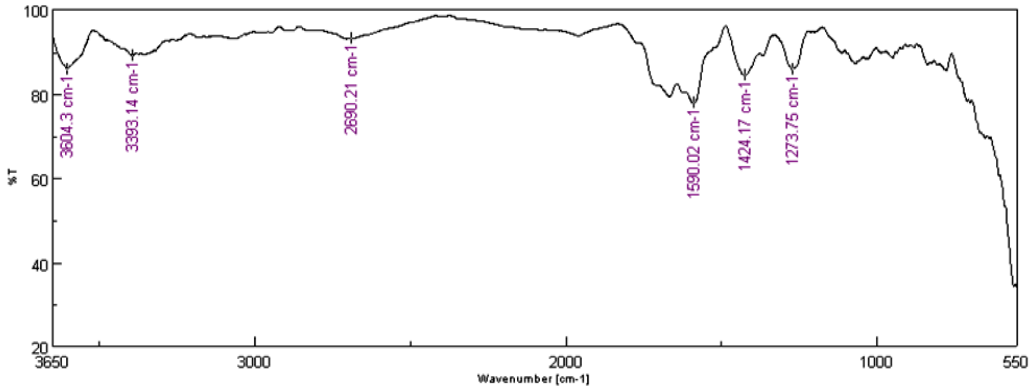

Figure 4: FTIR spectrum of Dichloromethane extract of *Aegle marmelos* L.

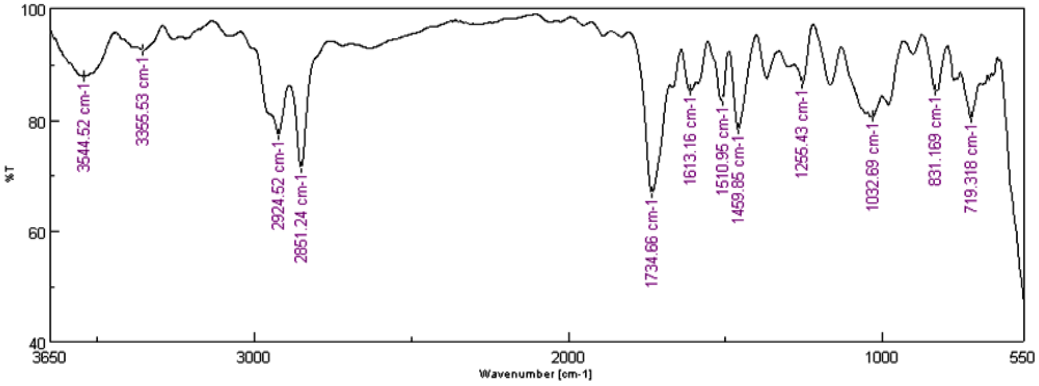

Figure 5: FTIR spectrum of Ethanol extract of *Aegle marmelos* L.

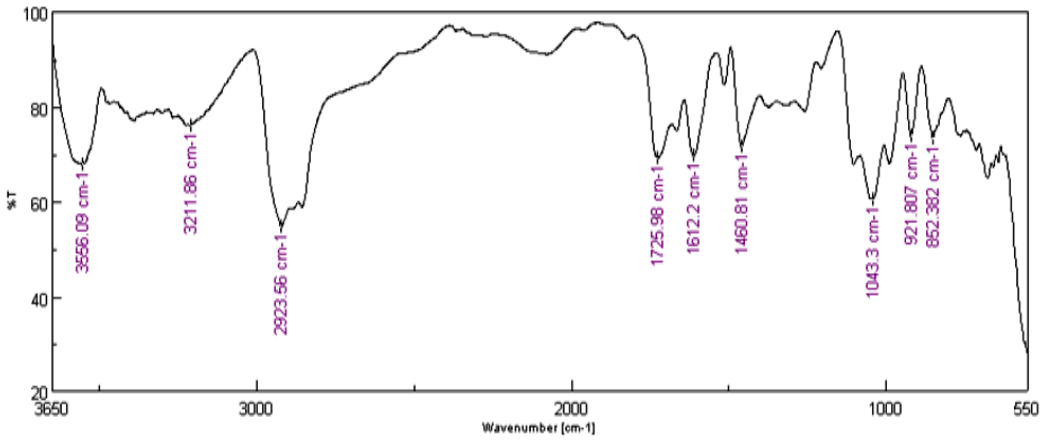

Figure 6: FTIR spectrum of Methanol extract of *Aegle marmelos* L.

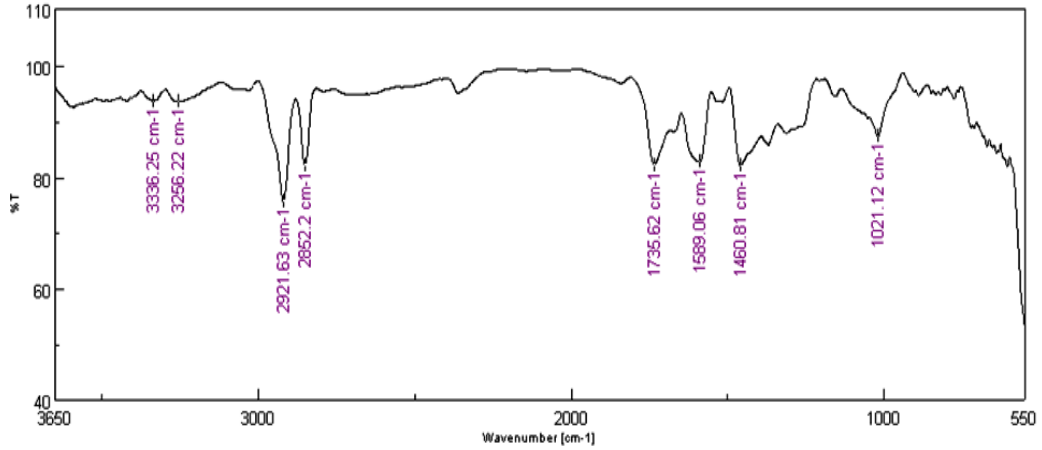

Figure 7: FTIR spectrum of Petroleum ether extract of *Aegle marmelos* L.

Fourier Transform Infrared Spectroscopic Analysis

The spectroscopic technique has become an analytical and most powerful tool for the qualitative and quantitative analysis of pharmaceutical and biological materials [1]. The Fourier Transform Infrared Spectroscopy (FTIR) was used to identify functional groups of the chemical constituents present in plant extracts based on the peak values in the IR region [50]. Results of FTIR spectroscopic analysis in the different solvent extracts of *Aegle marmelos* L. leaves revealed structural information of various functional groups were illustrated in figure no. 1 to 7. The absorption bands and the wavenumber (cm-1) of prominent peaks obtained from absorption spectra were depicted in **Table 2**.

In the present study, the aqueous extract of *Aegle marmelos* L. showed characteristic absorption bands at 3342 and 3257.18 cm-1 revealed the presence of the primary amines (N-H stretch). The peaks at 2851.2 and 2922.5 cm-1 refer to the presence of alkanes (C-H stretch).The strong absorption bands observed at 1734.6 and 1460.8 cm-1 corresponding to C=O stretch and C-O stretch representing aldehydes and a carboxylic acid group. The peaks at 1605.4, 1514.8 and 721.2 cm-1 refer to the presence of alkenes and aromatic compounds (C=C bend).The bands at 1252.5 and 1034.6 cm-1 which indicates the presence of esters and ethers assigned to the C-O stretching (**Figure 1**). The acetone extract exhibited the broad peaks at 3570.5, 3362.2, 2919.7, 2852.7, 1607.3, 1456.9 and 1052.9 cm-1 represents the presence of functional groups such as alcohols (O-H stretch), alkanes (C-H stretch), alkenes (C=C stretch), aromatic amines (C-N stretch) and carboxylic acids, esters and ethers (C-O stretch). The peak at 1730.8 cm-1 denotes the aldehydes and carbonyl group (C=O stretch) (**Figure 2**).

FTIR spectroscopic analysis of ethyl acetate extract (*Aegle marmelos* L. leaves) was shown in (**Figure 3)**. The more intense bands were observed at 3541.6, 3261, 3079.7, 2923.5, 2852.2, 1729.8, 1613.1, 1511.9, 1458.8, 1255.4, 1168.6, 980.6, 832.1 and 720.2 cm-1 corresponding to alcohols (O-H stretch), alkanes (C-H stretch), alkenes(C=C stretch & C=C bend), aromatics(C=C bend), carboxylic acids(C=O stretch), amines (N-H stretch), nitro compounds(N=O stretch), ethers(C-O stretch) and halides (C-F). The characteristic absorption bands at 3604.1, 3393.1, 2690.2, 1590, 1273.7 cm-1, which corresponds to O-H stretch, N-H stretch, C-H stretch and C=C stretch representing alcohols, amides, amines, aldehydes, carboxylic group and alkenes in dichloromethane extract (**Figure 4**). The ethanol extract showed peak intensities at 3544.5, 3355.5, 2924.5, 2851.2, 1734.6, 1613.1, 1510.9, 1459.8, 1255.4, 1032.6, 831.1, 719.3 cm-1 indicates the presence of functional groups such as alkanes (C-H stretch & -CH_2 bend), aldehydes (C=O stretch), alkenes(C=C stretch &

C=C bend) , alcohols(O-H stretch), ethers (C-O stretch) and nitro compounds (N=O stretch) (**Figure 5**).

The broad peaks observed in the methanol extract of *Aegle marmelos* L. at 3556.09, 3211.8, 2923.5, 1725.9, 1612.2, 1460.8, 1043.3, 921.6 and 852.3 cm-1 represents the presence of functional groups like alcohols(O-H stretch), carboxylic acid(O-H stretch), aromatics (in-rings) (C=C bend), alkanes (-CH_2 bend), alkenes (C=C bend), aliphatic amines(C-N stretch) and carboxylic acids, esters, ethers(C=O stretch & C-O stretch) (**Figure 6**). The petroleum ether extract showed the absorption bands occurring at 3336.2 and 3256.2 cm-1 refers to alcohols (O-H stretch). The major peaks at 2921.6, 2852.2 and 1460.8 cm-1 indicate the presence of alkanes (C-H stretch & -CH_2 bend) and carboxylic acid group (O-H stretch). The peaks observed at1735.6, 1589 and 1021.1 cm-1 assigned to the C=O stretch, C=C bend and N-H stretch which corresponds to the presence of esters, aromatics and amines (**Figure 7**).

FTIR gives the broad peaks observed around 3373-3422 cm-1 may be due to the presence of bonded N-H/C-H/O-H stretching of amines and amides [51]. The major peaks observed at near 2848 cm-1 represent C-H symmetric stretching of the methylene group in aliphatic compounds [52, 53]. In the present results, in all extracts of *Aegle marmelos* L. leaves, the peak intensities at 2924.5, 2851.2, 2852.2, 2923.5, 2919.7, 2921.6 cm-1 revealed the presence of alkanes, methylene, methyl ether, methoxy and aldehyde groups respectively.

Gaurav Kumar *et al* (2010) analyzed the methanolic and aqueous leaf extracts of *Bauchinia racemosa* by FTIR spectroscopy revealed the presence of flavonoids, saponins, tannins, proteins, phenolic compounds and carbohydrate as major functional groups [54]. The FTIR spectroscopic studies revealed the presence of alcohol, alkanes, aromatics, alkyl halide, carboxylic acid and halogen compounds from methanolic leaf extract of *Solanum torvumto* studied by [55]. FTIR analysis of the ethanol extract of *Ichnocarpus frutescens* revealed the presence of functional groups of amines, amides, amino acids, carbonyl compounds, carboxylic acids, organic hydrocarbons and halogens [56]. Florence and Jeeva (2015) also worked the ethanolic leaf extract of *Gmelina asiatica* confirm the presence of alkanes, carboxylic acids, alkyl halide, alcohol and halogen compounds through the FTIR analysis [57]. Ragavendran *et al* (2011) analyzed the FTIR spectral analysis of *Aerva lanata* leaf extracts and reported that the presence of various functional groups like amines, carboxylic acids, halogens, polysaccharides, sulphur derivatives and organic hydrocarbons are present in the extracts [58].

Manoj and Ragothaman (1999) reported that the strong absorption bands occurring at 3419, 2853, 1633, 1421, 1260, 1073, 816 and 635cm-1 corresponding to O-H, N-H, C-H, C-O and C-CI/C-CS stretching or bending vibrations representing the presence of ethers, nitrates, alkenes, halogen compounds [59] and also reported that the characteristic absorption bands were observed around 770-922 cm-1 may be due to presence of the carbohydrates [60]. There is no absorbance in between the region 2220-2260 cm-1 indicates that no cyanide groups in all extracts. This result exhibit all extracts of *Aegle marmelos* L. leaves do not contain any toxic substances.

FTIR technique was used for assessing the type of organic and inorganic components in plants. The FTIR analysis study has confirmed the presence of functional groups, which may be important in the synthesis of bioactive phytoconstituents. Detection of hydroxyl groups is an indication of flavonoids, alcohols and phenolic compounds [61]. *Aegle marmelos* L. is an aromatic plant that is confirmed in present research by the detection of aromatic functional groups. The flavonoids are commonly known as catechins, which are comprised of aromatic rings and hydroxyl groups possessing strong antioxidant activities [62]. Some proteins and lipids have asymmetric stretching due to C-H vibration, which was detected in all extracts of *Aegle marmelos* L. leaves.

In the present study, the very strong absorption band observed at the 3393.1, 3355.5, 3257.1, 3342, 3336.2 cm-1, which indicates the presence of alkaloids due to N-H stretch representing primary and secondary amines. The saponins were found to be present due to the presence of C=O stretch at 1729.8, 1734.6, 1730.8, 1725.9 cm-1 and C-O stretch at 1458.8, 1459.8, 1460.8, 1052.9 cm-1 as carboxylic acid anhydrides. The esters peak for C-O stretch at the 1255.4, 1252.5, 1052.9 cm-1 and C=O stretch at 1729.8, 1730.8, 1725.9, 1735.6 cm-1 were due to presence of steroids. Anthraquinones were present as aromatic ethers with C-O stretch at 1273.7, 1255.4, 1252.5 and 1043.3, 1021.1cm-1. The presence of quinines revealed the flavonoids, tannins, saponins and glycosides were present with O-H stretching and bending vibrations in all extracts of *Aegle marmelos* L. leaves. These findings indicate the presence of alkanes, alkenes, aldehydes, carboxylic acids, aromatics, amines, esters, alcohols, ethers, halides, carbonyl group and nitro compounds in leaves extracts of *Aegle marmelos* L. The stretches such as C-H, C=O, C=C,C-N, N-O and S-OR with the nearest range detecting the same functional groups reported by [63, 64]. From the results, it is confirmed that the presence of secondary metabolites like alkaloids, flavonoids, saponins, tannins, steroids, phenols and cardiac glycosides in the leaves of *Aegle marmelos* L.

CONCLUSION

The qualitative phytochemical analysis of the different extracts of *Aegle marmelos* L. leaves shows the presence of active constituents like alkaloids, saponins, flavonoids, amino acids, steroids, tannins, glycosides and phenolic compounds were screened and the results of FTIR analysis confirmed the presence of characteristic peak values with various functional groups such as alkanes, alkenes, amines, esters, ethers, aldehydes, carboxylic acids, aromatics, alkyl halides and nitro compounds. All these compounds relate to secondary metabolites and might be responsible for the various medicinal properties of *Aegle marmelos* L. Based on the results obtained

in the present study, it could be concluded that, the leaf extracts of *Aegle marmelos* L. contain medicinally important bioactive compounds. This indicates that leaves of *Aegle marmelos* L. can be useful for several diseases and phytoconstituents may act as potential sources of useful drug discovery in the future.

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CONFLICTS OF INTREST

There are no conflicts of interest.

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DATA AVAILABILITY

Not declared.

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