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Evaluation of Active Phytochemicals in Methanol extract of leaves of *Aegle marmelos* Linn.

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Abstract: *Aegle marmelos (L.) is a spinous deciduous and aromatic tree of family Rutaceae with long, strong and axillary spines. The bael tree contains furocoumarins, including xanthotoxol and the methyl ester of lloimperatorin, as well as flavonoids, rutin and marmesin; a number of essential oils; and, among its alkaloids, a-argarine (allocryptopine), O-isopentenylhalfordinol, O-ethylhafordinol. Aegeline(N-[2-hydroxy-2(4-methoxyphenyl) ethyl]-3-phenyl-2-propenamide) is a constituent extracted from bael leaves. Aeglemarmelosine, molecular formula* $C_{16}H_{15}NO_2$ $[\alpha]^{27}_{D=}$ *+7.89° (c 0.20,* $CHCl_3$*), has been isolated as an orange viscous oil.*
In the present investigation, the bioactive compounds in the leaves of Aegle marmelos (L.) were identified using HPTLC and GC-Mass spectroscopic techniques. The results revealed that the aqueous powder leaf extract of Bael showed eleven different phytocomounds with with eleven different Rf values from 0.04 to 0.47. The aqueous leaf showed 16 different compounds with 16 different Rf values from 0.06 to 0.51.
The methanol powder extract of leaf showed one Rf value more or less equivalent to Rf value 0.70 of marmelosin. The methanol leaf extract of leaf showed one Rf value more or less equivalent to Rf value 0.69 and 0.75 of marmelosin. GC-MS chromatogram of the methanolic extract of leaves of Bael showed thirty-four peaks of phytoconstituents. In all thirty-four, compounds were identified from the GC-MS analysis of a methanolic extract of Bael leaves exhibiting various phytochemical activities and might be responsible for various biological activities.

Key Words: *Aegle marmelos, Active phytochemicals, HPTLC, GC-Mass spectroscopy.*

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I. Introduction

Aegle marmelos (L.) is a spinous deciduous and aromatic tree of family Rutaceae with long, strong and axillary spines. This tree grows up to 18mt in height and thickness of tree is about 3- 4ft. Leaves are tri- to pentafoliate, leaflets are ovate and have typical aroma. Flowers are greenish white in colour and sweet scented. Fruits are large, woody, grayish yellow, 8- 15 celled and have sweet gummy orange coloured pulp. Seeds are compressed, oblong and numerous found in aromatic pulp.

Aegle marmelos (L.) Correa, commonly known as Bael, is a sacred tree for Hindu Religion, native to northern India, but is found widely throughout the Indian peninsula and in Ceylon, Burma, Thailand and Indo-China [1] (Bailey 1963). All parts of the tree viz. root, leaf, trunk, fruit and seed are used for treatment of many different diseases.

The constituents of *Aegle* are used in heart diseases [2] (Kakiuchi *et al*., 1991), inflammatory and wound healing [3] (Udupa et al. 1994). Leaves of *A. marmelos* have been reported as hypoglycemic effect [4, 5] (Santhoshkumari and Devi 1990; Sharma *et al*., 1996). The essential oil from the leaves of *A. marmelos* is known to exhibited antifungal properties [6, 7] (Renu et al., 1986; Rana *et al*., 1997). Besides the medicinal uses, this plant was also studied for their antimicrobial, antifungal and insecticidal properties [8, 9] (Satyal *et al*., 2012; Kumar *et al*., 2008). The effect of leaf extracts of *Aegle marmelos* was also studied against *Anopheles subpictus* in their oviposition deterrent, ovicidal and repellent activities [10, 11] (Elango *et al*., 2009; Vineetha *et al*., 2009).

Higher and aromatics plants have been used traditionally in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts [12] (Hulin, *et al.*, 1998). Biologically active compounds from natural sources have always been a great interest for scientists working on infectious diseases [13] (Perumal and Ignacimuthu, 2000). Today a substantial number of drugs are developed from plants which are active against a number of diseases. The majority of these involve the isolation of the active ingredient found in a particular medicinal plant and its subsequent modification. In the developed countries 25 percent of the

medical drugs are based on plants and their derivatives [14] (Devi and Manoharan, 2011) and the use of medicinal plants is well known among the indigenous people in rural areas of many developing countries. In the past our ancestors have made new discoveries on the healing power of plants through trial and error. The medicinal plant therapy is based on the empirical findings of hundreds and thousands of years [15] (Fakim, 2006). *Aegle marmelos* Correa is commonly called as Bael in Hindi, and Bilva in Sanskrit. It belongs to the family Rutaceae. It is indigenous to India and is used in folk medicines. The Ayurvedic practitioners use almost all of their parts but the greatest medicinal value of its fruits [16] (Ariharan and Prasad, 2013).The leaves are used as astringent, laxative, febrifuge and expectorant. The leaves are useful in ophthalamia, inflammations, catarrh, diabetic and asthmatic complaints [17] (Chakraborty, 2012).The leaves are used for the heart and brain disorders.

The various phytochemicals from different parts of *Aegle marmelos* tree have already been investigated and isolated. Different phytochemicals have been isolated from the stem bark of *Aegle marmelos* such as marmesin–1"- α -L - rhamnopyranoside and 1, 5 -dihydroxy - 6 - methoxy -2 - methyl anthraquinone along with lupeol and β-sitosterol. The leaves of *Aegle marmelos* also yielded other chemical compounds such as Aegeline, Lupeol, Cineol, Citral and Eugenol. Moreover, Marmelosin, Luvangetin and Marmelide have been reported and isolated from fruits of *Aegle marmelos* [18] (Maity *et al*., 2009).

The bael tree contains furocoumarins, including xanthotoxol and the methyl ester of alloimperatorin, as well as flavonoids, rutin and marmesin; a number of essential oils; and, among its alkaloids, a-fargarine (allocryptopine), *O*-isopentenylhalfordinol, *O*- ethylhafordinol [19] (Rasadah Mat Ali *et al*., 2010). Aegeline (N-[2-hydroxy-2(4-methoxyphenyl) ethyl]-3-phenyl-2-propenamide) is a constituent extracted from bael leaves [20, 21]. (Chatham- Stephans *et al*., 2017; Abula *et al*., 2016). Aeglemarmelosine, molecular formula $C_{16}H_{15}NO_2$ $[\alpha]^{27}{}_{D} = +7.89°$ (*c* 0.20, $CHCl_3$), has been isolated as an orange viscous oil [22] (Laphookhieo, Surat, 2011).

Indian Medicinal plants are considered a vast source of several pharmacologically active principles and compounds, which are commonly used in home remedies against multiple ailmets [23, 24] (Biswas *et al*., 2002; Chatopadhyay *et al*., 2004). Bael (*Aegle marmelos* (L.) Correa is another Indian medicinal plant which has enormous traditional values against various diseases and many bioactive compounds have been isolated from this plant [25, 26] (Badam *et al*., 2002; Gupta and Tondon, 2004).

The different parts of Bael are used for various therapeutic purposes, such as for treatment of Asthma, Anaemia, Fractures, Healing of Wounds, Swollen Joints, High Blood Pressure, Jaundice, Diarrhoea Healthy Mind and Brain Typhoid Troubles during Pregnancy [27] (Saswatiparichha, 2004).

Aegle marmelos has been used as a herbal medicine for the management of diabetes mellitus in Ayurvedic, Unani and Siddha systems of medicine in India [28] (Kar *et al*., 2003), Bangladesh [29] (Lampronti *et al*., 2003) and SriLanka [30] (Karunanayaki *et al*., 1987). The unripe dried fruit is astringent, digestive, stomachic and used to cure diarrhea and dysentery. Sweet drink prepared from the pulp of fruits produce a soothing effect on the patients who have just recovered from bacillary dysentery.

Plant derived compounds have got an increasing interest throughout the world as they possess potent, less or no toxic pharmacological compound, economic viable, safer and more dependable [31] (Prashant *et al*., 2008). About 70 – 95% of the world population are relying on traditional medicines or traditional therapies where the whole or parts of plants is used as medicine [32] (Robinson, 2011). Drug resistances in microorganism have become an unsolvable problem and treating an infectious disease with the existing drugs is becoming less use. This situation, truly made researchers to discover drug from various sources, one such source is plant based drugs. *Aegle marmelos* is one such plants where all the parts of the plant are known to possess various pharmacologically active compounds. It has been found that methanolic, toluene, water and chloroform extract *of Aegle marmelos* possess antimicrobial activity against the plant pathogens of *Daucus carota*, *Capsicum* and *Pomegranata* sps [33] (Chavda *et al*., 2012). Methanolic extract of *Aegle marmelos* was reported to have antibacterial activity against *Bacillus* sps, *E.coli* and *Klebsiella* sps [34] (Poonkothai and Saravanan, 2008). Various separation and detection techniques like TLC, HPLC, GC-MS etc. to analyse compound present in *Aegle marmelos* have been standardized [18] (Maity *et al*., 2009). Compounds such as skimimianine, ageline, lupeole, citral and marmesinin from leaves, marmelosin luvangetin, aurapten, psoralen from fruit and faganine, marmemin from bark were identified from *A.marmelos* [35] (Suvimol *et al*., 2008).

The aim of present investigation is to identify bioactive compounds present in the aqueous and methanolic leaf extract of *Aegle marmelos* employing HPTLC and Gas Chromatography- Mass Spectroscopy technique.

II. Materials and Methods

HPTLC analysis of Leaves of *Aegle marmelos*

Active phytochemicals in leaves of Aegle marmelos were analyzed in aqueous and methanol extract usin HPTLC (High Performance Thin Layer Chromatography).

Leaf Powder extract: 5g of powder sample was taken in two round bottom flasks each containing 100 ml of de-ionized water and 100 ml of methanol. They were then boiled at 100^0C for 1 hour.
The solution was then filtered using Whatman No.1 filter paper and both extracts were stored separately at 4^0C.

Leaf extract: 5g of material sample (leaf) was taken in two round bottom flasks each containing 100 ml of de-ionized water and 100 ml of methanol. They were then boiled at 100^0C for 1 hour. The solution was then filtered using Whatman No.1 filter paper and both extracts were stored separately at 4^0C.

Preparation of marmelosin standard: Stock solution was prepared by dissolving 1mg of marmelosin in 1ml of methanol. From this working standard has been prepared with the concentration of 5µg/ml.

HPTLC analysis: 2µ l of aqueous and methanol extracts (Powder extract and Material extract) of various parts of *Aegle marmelos* and 2µ l of marmelosin (marker compound) were applied to plates as 8.0mm bands on 20 cm x 10cm precoated silica gel 60 F254 TLC plates with layer thickness 0.2 mm using micro syringe by means of Linomat 5 applicator. Plates were developed in twin trough chamber using the mobile phase (Toluene: Ethyl acetate (7.5: 2.5)) for different extracts up to 80 mm distance. The plates were dried and scanned using Camag Reprostar 3 TLC Scanner at 254 and 366 nm.

Gas Chromatography-Mass Spectrometry analysis

The active phytochemicals in the methanol extract of leaves of Aegle marmelos were assayed by Gas Chromatography coupled with Mass Spectroscopy (Model; QP 2010 Plus, Shimadzu, Tokyo, Japan) equipped with a VF-5ms fused silica capillary column of 30m length, 0.25mm diameter, and 0.25µm film thickness. The column oven temperature was programmed from 80°C to 310°C for 2°C min-1. Ionization of the sample components was performed in electron impact mode (EI, 70 eV). The temperature of the injector was fixed to 270°C and one of the detectors to 230°C. Helium (99.9995% purity) was the carrier gas fixed with a flow rate of 1.21 ml min-1. The mass range from 40-650 m/z was scanned at a rate of 3.0 scans/s. 2.0 µl of the methanolic extract of *Aegle marmelos* was injected with a Hamilton syringe to the GC-MS manually for total ion chromatographic analysis in split injection technique. Total running time of GC-MS was 56 mins. The relative percentage of the each extract constituents was expressed as a percentage with peak area normalization.

The bioactive compounds of methanol extract were identified by comparing their retention indices and patterns of mass spectra with reference to Wiley Registry of Mass Spectral Data's, New York (Wiley 8) and Fatty Acid Methyl Esters Library version 1.0 (FAME library) sources. The results obtained have been presented in Table-1 – 4; Fig- 1, 2, 3 and 4.

Table- 1: Rf value and area under the curve for aqueous powder extract of leaves of *Aegle marmelos* at 366nm

Extracts of *Aegle marmelos*	Rf values	Areas under curve at 366nm
Aquous powder extract	0.04	248.5
	0.09	125.3
	0.21	493.4
	0.32	1223.9
	0.39	1169.1
	0.47	712.2

Table- 2: Rf value and area under the curve for aqueous extract of leaves of *Aegle marmelos* at 366nm

Extracts of *Aegle marmelos*	Rf values	Areas under curve at 366nm
Aquous leaf extract	0.06	285.5
	0.08	392.7
	0.12	273.0
	0.19	669.1
	0.22	526.0
	0.26	453.0
	0.34	586.5
	0.39	454.2
	0.49	336.9

Table- 3: Rf value and area under the curve for marker and methanol powder extract of leaves of *Aegle marmelos* at 366nm

Extracts of *Aegle marmelos*	Rf values	Areas under curve at 366nm
Methanol powder extract	**0.09**	**77.6**
	0.18	**127.9**
	0.28	**218.3**
	0.38	**175.9**
	0.44	**149.1**
	0.57	**209.6**
	0.72	**125.8**
	0.82	**115.2**
STD (Marmelosin) marker		
	0.70	**621.0**
	0.76	**740.0**

Table- 4: Rf value and area under the curve for marker and methanol extract of leaves of *Aegle marmelos* at 366nm

Extracts of *Aegle marmelos*	Rf values	Areas under curve at 366nm
Methanol extract	**0.18**	**317.0**
	0.28	**349.6**
	0.36	**325.6**
	0.40	**709.4**
	0.55	**510.8**
	0.80	**7976.4**
STD (Marmelosin) marker	**0.70**	**621.0**
	0.76	**740.0**

(a) (b)

Figure- 1: HPTLC profile of aqueous extract of Aegle marmelos at 366nm. (a) Powder leaf extract; (b) aqueous leaf extract; Horizontal lines indicates the identical compounds with same Rf values.

(a) (b)

Figure- 2: HPTLC profile of methanol extract of leaf of Aegle marmelos at 366nm. (a)Methanol powder extract (b) methanol leaf extract.

Figure- 3: HPTLC profile of Standard Marmelosin

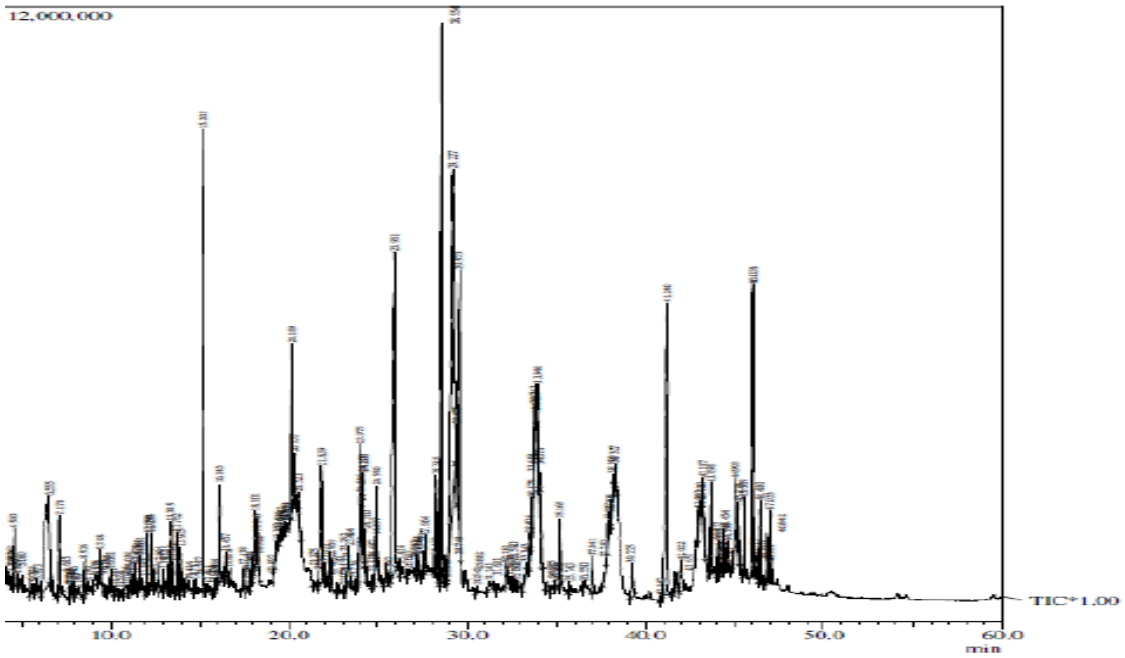

Figure -4: GC-MS chromatogram of methanolic extract of leaves Bael (*Aegle marmelos*)

Table- 5: Characterization of active phytochemicals identified in methanolic leaf extract of *Aegle marmelos*

S.No.	R. T	Compound identified	**Molecular formula**	Molecular weight	Peak area (%)	Nature of compound	Known activity
1.	7.178	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	C6H8O4	144	1.11	Flavonoid fraction	Antimicrobial, Anti inflammatory, antiproliferative
2.	6.555	1-Butanol, 3-methyl-, acetate	C7H14O2	130	5.32	Alcoholic compound	Antimicrobial
3.	12.09	2,3 Dioxabicyclo[2.2.2] oct-5-ene, 1-methyl-4-(1-methylethyl)-(Limonene dioxide 1)	C10H16O2	168	0.53	Terpene	Antimicrobial activity
4.	13.385	Bicyclo[3.1.1]heptane-2,3-diol, 2,6,6-trimethyl (2,3-Pinanediol)	C10H18O2	170	0.97	Terpene	antimicrobial activity
5.	13.762	2-Cyclohexen-1-one, 4-hydroxy-3-methyl-6-(1-methylethyl)-	C10H16O2	168	0.63	long-chain fatty alcohol	Antibacterial
6.	15.203	1-Dodecanol	C12H26O	186	4.83	long-chain fatty alcohol	Antibacterial
7.	16.143	Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl (BHT)	C15H24O	220	0.87	Aromatic acid ester	Antimicrobial, antioxidant activity
8.	16.457	Benzoic acid, 4-ethoxy-, ethyl ester	C11H14O3	194	0.33	Aromatic acid ester	Antimicrobial Preservative
9.	18.101	2-Propanol, 1,1'-[(1-methyl-1,2-ethanediyl)bis(oxy)]bis-(Tripropylene glycol)	C9H20O4	192	0.87	Fatty acid esters	Antimicrobial activity
10.	20.189	1-Tetradecanol, acrylate	C17H32O2	268	1.46	Fatty acid esters	Anti-inflammatory, Antimicrobial
11.	20.523	1,3,4,5 Tetrahydroxy-cyclohexanecarboxylic acid (Quinic acid)	C7H12O6	192	0.39	Aromatic acid	antimicrobial activity, anti-inflammatory
12.	21.839	Tetradecanoic acid (Myristic acid)	C14H28O2	228	2.00	Fatty acid	Antifungal, Antioxidant, cancer preventive, nematicide, hypercholesterolemic, Lubricant
13.	22.070	2(4H)-Benzofuranone	C11H16O3	196	0.26	Triterpene	Antimicrobial

		5,6,7,7a-tetrahydro-6-hydroxy-4,4,7a-trimethyl					
14.	22.262	1-Heptadecanol (1-Eicosanol)	C17H36O	256	0.33	Aliphatic alcohol	Antimalarial, antifungal, Antioxidant
15.	22.409	1,3-Cyclohexadiene, 2-methyl-5-(1-methylethyl)-(1-Phellandrene)	C10H16	136	0.23	Monoterpene	Antibacterial
16.	22.736	1,6-Octadiene, 7-methyl-3-methylene (beta.-myrcene)	C10H16	136	0.13	Monoterpene	Antibacterial
17.	23.404	2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-, methyl ester (Cinnamic acid, 4-hydroxy-3-methoxy-, methyl ester)	C11H12O4	208	0.66	Aromatic methyl esters	Antimicrobial, antioxidant, antiviral
18.	23.819	Pentadecanoic acid	C15H30O2	242	0.08	Fatty acid	Antibacterial
19.	24.897	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)	C20H40O	296	0.32	Diterpene	Antimicrobial, anticancer, anti-inflammatory, anti-diuretic,
20.	24.986	hexadecanoic acid, methyl ester (Palmitic acid methyl ester)	C17H34O2	270	0.72	Fatty acid methyl ester	Antioxidant, hypocholesterolemic, nematicide, pesticide, anti androgenic flavor, hemolytic, 5-Alpha reductase inhibitor
21.	24.951	Pentadecanoic acid	C15H30O2	242	7.99	Saturated fatty acid	Antimicrobial
22.	27.266	9-Octadecenoic acid	C18H34O2	282	0.31	Unsaturated fatty acid	Antibacterial
23.	27.664	Heptadecanoic acid	C17H34O2	270	0.39	Saturated fatty acid	Antimicrobial
24.	28.298	9,12,15-Octadecatrienoic acid, methyl ester (Linolenic acid, methyl ester)	C19H32O2	292	1.07	Fatty acid methyl ester	Antibacterial, anticandidal, antiinflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge Antihistaminic, Antiarthritic, Anticoronary, Antieczemic Antiacne, 5-Alpha reductase inhibitor Antiandrogenic
25.	28.554	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl (Phytol isomer)	C20H40O	296	6.37	Diterpene	Antimicrobial Anti-inflammatory Anti cancer Diuretic
26.	29.227	Cis-9-Hexadecenal	C16H30O	238	11.40	Aldehyde	Antimicrobial
27.	29.573	Octadecanoic acid (Stearic acid)	C18H36O2	284	4.06	Fatty acid	Antimicrobial
28.	41.240	Benzene, 1,2-dimethoxy-4-[[(4 methylphenyl)] sulfonyl methyl	C16H18O4 S	306	10.76	Aromatic sulfur compound	Antimicrobial
29.	44.155	Cholest-5-en-3-ol (3.beta.)-	C27H46O	386	0.24	Steroidal compound	Antibacterial
30.	45.238	Ergost-5-en-3-ol, (3.beta.)-	C27H46O	386	0.50	Steroidal compound	Antibacterial, anti-inflammatory effects
31.	45.509	Stigmasta-5,22-dien-3-ol	C29H48O	412	0.63	Steroidal compound	Antioxidant, antibacterial activity, antiinflammatory,antiarthritic antiasthma, diuretic
32.	46.038	Stigmast-5-en-3-ol, (3.beta.)-	C29H50O	414	3.08	Steroidal compound	Antimicrobial, antioxidant, antiinflammatory, antiarthritic, antiasthma, Diuretic
33.	47.033	Vitamin E	C29H50O2	430	0.58	Fat soluble	Antioxidant and

							Antimicrobial activity, Analgesic, Antidiabetic Antiinflammatory, Antidermatitic, Antileukemic, Antitumor, Anticancer, Hepatoprotective, Antispasmodic
34.	44.454	alpha -Tocopherol	C29H50O2	430	0.26		Anti-inflammatory, antioxidant, antimicrobial, radical scavenging, antispasmodic

III. Results

The results of the preliminary phytochemical studies confirmed the presence of alkaloids, cardiac glycosides, terpenoids, saponins, tannins, flavanoids and steroids in six solvent extracts of leaves of *A. marmelos* [36] (Snehlata et al., 2018). The aqueous powder leaf extract of Bael showed eleven different phytocomounds with with eleven different Rf values from 0.04 to 0.47 (Table- 1; Fig- 1). The aqueous leaf showed 16 different compounds with 16 different Rf values from 0.06 to 0.51 (Table- 2; Fig- 2).

The methanol powder extract of leaf showed one Rf value more or less equivalent to Rf value 0.70 of marmelosin (Table- 3) The methanol leaf extract of leaf showed one Rf value more or less equivalent to Rf value 0.69 and 0.75 of marmelosin (Table- 4; Fig- 3).

GC-MS chromatogram of the methanolic extract of leaves of Bael showed thirty-four peaks of phytoconstituents (Table- 5). All the thirty four compounds identified exhibit various phytochemical activities and might be responsible for various biological activities. The retention time and percentage peak of various bioactive compounds are presented in Table- 5.

IV. Discussion

The phytocompounds with same Rf value found in both powder and material aqueous extract indicates that they are same compounds. The marker compound marmelosin is present in all parts of the plant and its concentration is high. Also, the Rf of other phytocompounds of methanol extracts which are near to marmelosin range may be other compounds under the class coumarins. Therefore, it is evident that coumarins are largely present in all both aqueous and methanol extracts of *Aegle marmelos*. The present findings are in agreement with the works of [37, 38, 40]Nirupama *et al*., (2012); Bhyan *et al*., (2009); Shailajan *et al*., (2011); Narayan and Chanotia (2009) who also reported the same phytochemicals in different solvent extracts of leaves of *Aegle marmelos*.

Diana Victoria *et al*., (2014) [41] separated different compounds in the ethanol extract of leaves of Aegle marmelos by thin layer chromatography using Chloroform as the mobile phase and revealed the presence of 13 bands with Rf value ranged from 0.8 - 6.2. TLC profiling of ethanol extract of *Aegle marmelos* showed three major bands in long UV and iodine sprayed plates with Rf value of 0.72, 0.70 and 0.58.

The compounds present in the fractions were Alpha-pinene (Rf/mins 3.958), D-Limonene (Rf/mins 5.614), Tetradeconoic acid (Rf/min15.635), 1, 2 benzene di carboxilic acid (Rf/min17.726), Octadecanoic acid (Rf/mins 19.541), Di-n-octyl phthalate (Rf/mins 22.766) and Squalene (Rf/min24.784). Alpha-pinene (mono terpene) and Squalene (triterpene) were also found. Terpenes are phenolic compounds which exhibits antibacterial and antifungal activity (Habtemariam *et al*., 1993). Squalene, a triterpene is found to possess chemopreventive activity against colon cancers (Rao *et al*., 1998) [42].

The major phyto-constituent present in the leaf extract were 1-Dodecanol (4.83), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-(1.11), 2,3Dioxabicyclo[2.2.2]oct-5-ene,1-Methyl-4-(1-Methylethyl)-(Limonene dioxide1)occupying two peak areas i.e. (0.53) and (0.40)., Bicyclo[3.1.1] heptane-2,3-diol, 2,6,6-trimethyl (2,3-Pinanediol)(0.97), 2-Cyclohexen-1-one, 4-hydroxy-3-methyl-6-(1-methylethyl)-(0.63,0.41,0.17), Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-(BHT) (0.87), Tetradecanoic acid (Myristic acid) (2.00), 2(4H)-Benzofuranone 5,6,7,7A-Tetrahydro-6-hydroxy-4,4,7a-trimethyl,1,3-cyclohexadiene,2-methyl-5-(1methylethyl)- (1-Phellandrene), 2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-, methyl ester (Cinnamic acid, 4-hydroxy-3-methoxy-, methyl ester), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol commonly known as phytol a diterpene has significant antimicrobial properties against many bacterial strains [43] (Bharathy *et al.*, 2012). 9,12,15-Octadecatrienoic acid, methyl ester (Linolenic acid, methyl ester) showing antibacterial and anticandidal activity, 2-Hexadecen-1-ol, 3,7,11,15-Tetramethyl (Phytol isomer) (6.37), Octadecanoic acid (Stearic acid) (4.07), Benzene, 1,2-dimethoxy-4-[[(4 methylphenyl)sulfonyl]methyl (10.76), fatty alcohols such as Ergost-5-en-3-ol, (3.beta.) (Campesterol), Stigmasta-5, 22-dien-3-ol, Stigmast-5-en-3-ol, (3.beta.)- may be synergistically responsible for the antimicrobial activity. The present findings gain support from the study of

[44] Farina Mujeeb *et al*., (2014) who have analyzed similar phytochemicals in leaf extracts of eighteen different varieties of *Aegle marmelos*.

The different phytocompounds responsible for bioactivity have been identified and characterized in different medicinal plants. 4H-Pyran-4-one, 2,3-dihydro-3, 5-dihydroxy-6- methyl-, a potent anti-inflammatory and antioxidant compound, possessed antibacterial activity in *Barleria prionitis* (Linn.) rhizome.Along-chain fatty alcohol, 1-Dodecanol,was reported with highest antibacterial activity against *Staphylococcus aureus* [45] (Togashi *et al*., 2007). Phenol, 2,6-bis (1,1-dimethylethyl)-4- methyl commonly known as Butylated hydroxytoluene (BHT), an antioxidant, has also demonstrated marked antimicrobial activity inhibiting or decreasing the growth of gram-positive bacteria at a higher degree than the gram-negative bacteria belonging to the family Enterobacteriaceae [46] (Turcotte and Saheb, 1978). Derivatives of cinnamic acid such as esters, amides, acids, and hydrazides have been reported to have antibacterial, antifungal, and antiviral properties [47] (Sova, 2012). 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7atrimethyl, a bioactive compound possessing the properties such as analgesic, antidiabetic, antibacterial, and antifungal, was identified from the methanolic fractions of the *Azadirachta indica* [48] (Moorthy and Boominathan, 2011). The extract contained (0.23%) of 1, 3-Cyclohexadiene, 2-methyl-5-(1-methylethyl), commonly known as 1-phellandrene. Stigmasterol has been reported earlier as a strong antioxidant having antibacterial activity against multidrug resistant mycobacteria [49, 50] (Hamden *et al*., 2011; Navarro Garcia *et al*., 2011). Several bioactive compounds, namely, Marmin [49] (Hamdan et al. 2011) and Marmelosin coumarin derivatives [51] (Ram *et al*., 2012) andAegeline, an alkaloid, have been previously reported from *Aeglemarmelos* [52] (Nugroho *et al*., 2011). However, the flavonoids and phenolics present in significant amount in this plant are still unexplored. The preferential quantity of these compounds in the methanolic extract of PantAparna as revealed by the present study directed to focus towards the purification and characterization of potential compound from this variety.

IV. Conclusions

Medicines derived from plants have made immense contribution towards the betterment of human health and act as a source of inspiration for novel drug compounds. From the above research it can be concluded that this plant has immense potential to be used in the area of pharmacology and as a prospective source of valuable drugs. Due to the presence of various compounds that are essential for good health, it can also be used to improve the health status of society. The extracts showed a significantly high antibacterial activity against the microorganisms. The data clearly depicts the presence of compounds used for treating various bacterial diseases, indicating its use in the traditional system of medicine since ancient times. Further, the broad spectrum activity of aqueous, methanol, and aqueous: ethanol extracts proves to be encouraging in the development of novel antimicrobial formulation in the near future. A spectrum of compounds showing strong antibacterial, antioxidant, and anti-inflammatory activities was revealed by the GC-MS analysis of the methanolic extract of Aegle marmelos.

Antimicrobials derived from plants possess vast curative properties since they have fewer side effects as compared to synthetic antimicrobials drugs. Aegle marmelos is of utmost importance for ethnobotanical purposes, and it has been placed in the priority list of thirty-two medicinal plants by The National Medicinal Plants Board of Govt. of India [53] (Kala, 2006). The present study contributes to the current knowledge of presence of various phytochemical active compounds in leaves of Aegle marmelos possessing significant broad spectrum antibacterial efficacy. Further fractionation and purification will elucidate the potential compound, which is a pressing need because of the upcoming resistance of the currently available antibiotics.

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