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PHYTOCHEMICAL STUDY AND GC-MS ANALYSIS OF BAEL (*AEGLE MARMELOS*) FRUIT PULP

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ABSTRACT: Bael fruit (*Aegle marmelos* (L.) Correa) is an attractive and characteristically sweet aroma fruit, native to Southeast Asia, known to be a good source of natural antioxidants and bioactive compounds. To identify the potential sources of these bioactive compounds, extracts of *Aegle Marmelos* fruit were assessed with regard to their total phenolics and flavanoid content. Antioxidant activity of extracts in different solvent systems (methanol, ethanol and distilled water). Was also studied, in methanol extract the phenolics and flavanoide content of *Aegle Marmelos* fruit was 81.46 mg tannic acid equivalents /g fresh weight (TAE/g fw) and 21.68 mg rutin equivalents/ g fresh weight (RE/g fw) respectively. The effect of different solvents on the antioxidant activity of *Aegle Marmelos* was measured, methanol extract exhibited highest free radical scavenging activity (2.256 ± 0.14%), Ferric reducing 0.026± 0.12 Mg/AAE/g fw and ferrous ion chelating (59.60 ± 0.09 %) activities. The study was extended to study the volatile compounds in bael fruit pulp were analyzed by using the GC-MS techniques by using methanol, ethanol and petroleum ether extracts. In petroleum ether extract 21 compounds were identified as compared to the methanol (12) and ethanol (10) extract. In this extract the dominant compounds are nanocasene and hexocasene.

KEYWORDS: *Aegle marmelos*, phytochmicals, GC-MS analysis.

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1.INTRODUCTION

Bael fruit (*Aegle marmelos* (L.) Correa) is a tropical fruit native to Southeast Asia and belongs to the Rutaceae family. It is grown throughout India as well as in Sri Lanka, Pakistan, Bangladesh,

Burma, Thailand, and most of the Southeast Asian countries [1]. In Thailand, it is commonly found growing in many regions, especially the lower north and central part consisting of Phichit, Prachin Buri, and Phitsanulok provinces. There are no standard names for bael fruit cultivars. The general cultivars known in Thailand are locally "Matoom Kai" (in Thai language "Matoom" means bael fruit and "Kai" refers to cultivar). It is usually used for household consumption and traditional medicine, but some are planted for trade in particular regions [2]. The Bael fruit pulp contains many functional and bioactive compounds such as carotenoids, phenolics, alkaloids, coumarins, flavonoids, terpenoids, and other antioxidants which may protect us against chronic diseases [3].Therefore, bael fruit may indicate that it is one of the importantplants used for indigenous traditional medicine. There are innumerable references of its uses intraditional medicine [4] [5] [6] [7]. The uses of bael fruit in aspects of food have many forms in each country. For example, the ripe fruit is consumed fresh and also prepared as squash, sherbet and jam in India [8].The results of this study can add to the scientific literature and useful application from bael fruit for producers and consumers to be aware of the importance and utilization of this useful resource.

Phytochemical analysis

Aegle marmelos fruit pulp has been used as a remedy for gastrointestinal infections of human. This study reveals the antioxidant potentials of *Aegle marmelos* fruit pulp extracts. Standard methods were adopted to screen antioxidant and phytochemical nature of *A. marmelos* fruit pulp. Results of Phytochemical screening of the aqueous extract revealed the presence of steroid, terpinoids, saponins, tannis, lignin, flavonoids. Alcoholic extract showed the availability of alkaloids and devoid of saponin [9]. Plants is very useful, self-generating machines, producing a variety of useful bioactive products. Keeping in view this idea, the crude ethanolic extract and various fractions of *Aegl emarmelos* were screened for in vitro Antibacterial, Antifungal activity, Nutritional Evaluation and Phytochemical Screening. The plant edible fruit content nutrients such as crude protein, carbohydrates, crude fiber and ash content (2.40%, 29.40%, 3.20% and 2.12 %) and minerals as calcium, magnesium, potassium and phosphorus (0.85, 0.94, 1.12 and 0.50 mg/gm) respectively. The ethanolic fruit extracts of *Aegle marmelos* showed significant activity 14±1mm, 13±1mm and 13±1mm against Staphylococcus epidermidis, Shigellaflexneri and Enterobactergergoviae against food poisoning bacteria, and phytochemical screening for the presence of glycosides, flavonoids, phenols, resin and tannins. However, alkaloids were absent. This analysis revealed that, the fruits contained higher value of fat, protein, fiber and minerals as compared to the cultivated fruits with apple and mango. *Aegle marmelos* fruits contain sufficient amount of nutrients, required per day by a person. Consumption of fruits may promote general health and well-being as well as reduce the risk of chronic diseases. These findings confirms that the *Aeglemarmelos* may be potentional source for the formulation of nutraceuticals or natural foods [10]. *Aegle marmelos*, a plant indigenous to India has been used by the inhabitants of the Indian subcontinent for over 5000 years. The leaves,

bark, roots, fruits and seeds are used extensively in the Indian traditional system of medicine the Ayurveda and in various folk medicine to treat myriad ailments. Bael fruits are of dietary use and the fruit pulp is used to prepare delicacies like murabba, puddings and juice. Bael fruits are also used in the treatment of chronic diarrhea, dysentery, and peptic ulcers, as a laxative and to recuperate from respiratory affections in various folk medicines. Scientific studies have validated many of the ethno medicinal uses and reports indicate that the fruit possesses broad range of therapeutic effects that includes free radical scavenging, antioxidant, inhibition of lipid peroxidation, antibacterial, antiviral, anti-diarrheal, gastroprotective, anti-ulcerative colitis, hepatoprotective, antidiabetic, cardioprotective and radioprotective effects. For the first time, this review critically assesses the nutritional values, phytochemistry and preclinical pharmacological properties of the bael fruit. Attempts are also made at emphasizing the dietary and pharmaceutical potential of bael fruit that has been largely underutilized and neglected [11].

Antioxidant Study

To compare the antioxidant activity of the ethanolic extract of *Aegle marmelos* ripe and unripe fruit. *Aegle marmelos* is an important medicinal plant in India. Leaves, fruits, stems and roots of *A. marmelos* have been used in ethno medicine to exploit its medicinal properties including astringent, antdiarrheal, antidysenteric, antipyretic and anti–inflammatory activities. This study covers the antioxidant activity and free radical scavenging activity. From the present study it was identified that the enzymic antioxidants except glutathione peroxidase were increased in ripe fruit when compared to unripe fruit extract. Where as in non enzymic antioxidants reduced glutathione is increased in unripe fruit and ascorbic acid is decreased in unripe fruits. The percentage of free radical inhibition is also increased in unripe fruit when compare to ripe fruit [12]. Total content of phenol and flavonoid was quantitatively estimated in different parts of *A. marmelos*. The total phenolic contents varied from 9.8367 ± 0.0235 to 1.7281 ± 0.049 mg g-1. Total flavonoid contents were between 8.248 ± 0.029 to 1.087 ± 0.002 mg g-1. Free radical scavenging activity of different extracts was evaluated by using DPPH (1, 1 -Diphenyl- 2 -picrylhydrazyl) method. The highest free radical scavenging effect was observed in leaves with IC50 = 2.096μg ml –1. The effectiveness of radical scavenging activity of leaves extract was about 10 times greater than reference antioxidant butyrate hydroxy toluene (BHT). The greater amount of phenolic compounds leads to more powerful radical scavenging effect as shown by methanolic extract of *A. marmelos* leaves [13].

2. MATERIALS AND METHODS

Raw materials

Fresh bael fruits *Aegle marmelos* (L.) Correa were procured from local market of Beed district. Fully ripe bael fruits were chosen for this study because this usually consumed fresh and used as a main ingredient in many food products. The pulps were removed from the fruits, and were analyzed for phytochemical and volatile compounds. All chemicals and solvents used in this experiment were of

analytical grade.

Phytochemical analysis

Preparation of fruit extracts

Fruit extracts were prepared using three different solvent systems (methanol ethanol and petroleum ether).

Quantitative phytochemical analysis

Total phenols determination

Total phenolic contents (TPC) of the fruit extracts were determined using Folin-Ciocalteu method [14]. An aliquot of the extracts (0.125 ml was mixed with FolinCiocalteu reagent. Then 1.25 ml of saturated Na_2CO_3 solution was added and allowed to stand for 90 min at room temperature. Then, the absorbance was measured at 760 nm. A calibration curve was prepared using a standard solution of tannic acid (10 to 100 µg/ml, r2 = 0.998). Results were expressed on fresh weight (f_w) basis as mg tannic acid equivalents (TAE)/g of sample.

Total flavanoid determination

Total flavonoid contents (TFC) of the fruit extracts were analyzed according to the colorimetric method [15]. In brief, 1.5 ml of fruit extract was mixed with 1.5 ml of AlCl3 (2% w/v). It remained at room temperature for 10 min; the absorbance was measured at 368 nm. A calibration curve was prepared using a standard solution of rutin (10 to 100 µg/ml, r^2 = 0.964). The results were also expressed on a fresh weight basis as mg rutin equivalents (RE)/g of sample.

Determination of antioxidant properties

Preparation of fruit extracts

Fruit extracts were prepared using three different solvent systems (aqueous and methanol, ethanol).

DPPH radical scavenging activity

The antioxidant activities of the sample were assessed using 1, 1-diphenyl-2–picrylhydrazyl (DPPH) radical [16]. It is free radical with a purple colour and has a maximum absorption at 517 nm. The free radical scavenging activity is based on the discoloration of the compounds when reduced by a free radicals scavenger. About 100 µl of the sample at various concentrations was added to 2 ml of DPPH in solvent solution (60 µM) in a test tube and shaken vigorously. Incubate at 37^0C for 35 minutes in the dark. The absorbance of each solution was determined at 517 nm. The corresponding blank (control) reading was also taken [17]. The activity was expressed as percentage scavenging of the DPPH by the plant extracts and calculated as,

$$\%\text{DPPH radical scavenging} = \frac{\text{(Absorbance of control – Absorbance of sample)}}{\text{Absorption of sample}} \times 100$$

Ferric reducing antioxidant power assay

The ability to reduce ferric ions was measured using a modified version of the method described [18]. An aliquot (90 µl of extract was added to 2.7 ml of FRAP reagent) 10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10 mM 2,4,6- tris(2-pyridyl)-1,3,5-triazine (TPTZ) solution and 1 part of 20 mM FeCl3. 6H2O solution and the reaction mixture were incubated at 37°C for 15 min. After that, the absorbance was measured at 593 nm. A calibration curve was prepared, using an aqueous solution of ascorbic acid (100 to 1000 µM, r2 = 0.998). FRAP values were expressed on a fresh weight basis as micromoles of ascorbic acid equivalent per gram of sample.

Ferrous ion chelating activity

The chelating activity of the extracts for ferrous ions Fe2+ was measured [19]. To 0.5 ml of extract, 1.6 ml of deionized water and 0.05 ml of FeCl2 (2 mM) was added. After 30 s, 0.1 ml ferrozine (5 Mm) was added. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. After 10 min at room temperature, the absorbance of the Fe2+-ferrozine complex was measured at 562 nm. The percentage of chelating activity of the extract was determined.

Detection of volatile compounds

Preparation of fruit extract

Fruit extracts were prepared using three different solvent systems (methanol, ethanol and Petroleum ether).

GC-MS analysis

The solvent extracts were prepared with acetone, methanol and ethanol as per the procedure givens by Doughari [20] with slight modification. About 10 g of crushed flesh and wine sample was extracted with ethanol and kept on rotary shaker for 12 hr. the extracts were filtered, centrifuged at 5000 rpm for 10 min and supernatants were collected. The supernatants were filtrated and used for phytochemical analysis. Possible presence of active components in the solvent extracts of bael fruit pulp and prepared wine with acetone, methanol and ethanol were detected by GC – MS (Gas Chromatography Mass Spectroscopy). The GC – MS analysis of the samples was performed on a QP-2010 (Shimadzu) gas chromatography coupled with mass spectroscopy. The samples were injected into Rtx-5 MS capillary column (60 m length x 0.25 mm internal diameter x 0.25 µm film thickness). The carrier gas was helium at a flow rate 1.0 ml min^{-1}, linear velocity 25.6 cm sec^{-1}. The initial column temperature was 80ºC, then increased linearly at 10ºC min^{-1}, -280ºC and held for 11 min. the total run time was of 36.14 min. the temperature of the injection port was 280ºC and interface temperature was 290ºC. The injection volume was 1 µl. The mass spectra were recorded in Electron Ionization (EI) mode at 70º eV. Compound identification was accomplished by comparing the retention times with those of authentic compounds and fragmentation pattern, as well as with mass spectra in the NIST spectral library stored in the computer software (version 1.10 beta,

Shimadzu) of the GC – MS.

Statistical analysis

The data obtained are subjected to statistical analysis of variance (ANOVA) using complete randomized design. The critical difference at $P<0.05$ is estimated and used to find significant difference if any.

3. RESULTS AND DISCUSSION

Quantitative phytochemical analysis of bael fruit pulp

Phenolics and flavonoids are ubiquitously found in many plant sources including different vegetables, fruits and medicinal plants. Recently, the role of phenolic compounds in the prevention of free radical mediated diseases has become more important due to the discovery of the link between lipid peroxidation of low density lipoprotein (LDL) and arteosclerosis. They possess different antioxidant properties, which can be ascribed to a broad range of pharmacological activities. These compounds in general, act by quenching free radicals, inhibiting the activation of procarcinogens, or by binding carcinogens to macromolecules [21]. Methanolic extract also had highest TFC (21.68 mg RE/g fw) as compared to other solvents. The results of phytochemical analysis of *Aegle marmelos* (L.) fruit pulp. Were extracted with Different solvents were used successively with gradient polarity (Methanol, Ethanol) represents the qualitative analysis of the Phytochemicals of bael fruit pulp. The level of the phenolic compounds in the bael fruit pulp is as shown in Table 1. The total phenolic content (TPC) of the fruit extracts ranged from 76.28 to 80.59 mg TAE/g (fw) the total flavonoid content (TFC) of these fruits was also determined (Table 1.).

Table 1: Quantitative phytochemical analysis of bael fruit pulp

Solvents	Total phenolics (mg TAE/g fw)	Total flavonoid (mg RE/g fw) Aq
Aqueous	76.28 ± 5.07	15.20 ± 0.5
Methanol	81.46± 8.08	21.68 ± 0.2
Ethanol	80.59 ± 7.06	19.48 ± 0.3

Values expressed are means ± S.D. of three replicates.

Antioxidant properties of bael fruit

The antioxidant capacities of the plant extracts largely depend upon the compositions of the extracts and conditions of the test system. The antioxidant capacities are influenced by many factors that cannot be fully described with one single method. It is necessary to perform more than one type of antioxidant capacity measurement to take into account the various mechanisms of antioxidant action [22]. Therefore, in this study, three different solvents have been used to evaluate the antioxidant capacity of the bael fruit pulp; they are DPPH free radical-scavenging, ferric reducing antioxidant power (FRAP) assay and ferrous ion chelating activity assay.

DPPH radical scavenging activity

DPPH is a stable free radical and it accepts an electron or hydrogen radical to become a stable diamagnetic molecule which is widely used to investigate radical scavenging activity. The essence of DPPH radical scavenging assay is that antioxidants react with DPPH (deep violet color) and convert it to yellow colored 1, 1-diphenyl-2- picrylhydrazine. The degree of discoloration indicates the radical-scavenging potential of the antioxidant [23] [24]. In the present study, the fruits analyzed were able to decolorize DPPH and the free radical scavenging activity was expressed as the percentage decolorization. The DPPH free radical scavenging activity of the plant extracts are shown in Table 2.Ethanol extracts showed highest DPPH free radical scavenging activity followed by methanolic and aqueous extracts. This high scavenging property of ethanolic extract may be due to hydroxyl groups existing in the phenolic compounds' chemical structure that can provide the necessary component as a radical scavenger [25].

Ferric reducing antioxidant power

FRAP assay measures the reducing potential of antioxidant. Antioxidant compound which act as a reducing agent exert its effects by donating hydrogen atom to ferric complex and thus, break the radical chain reaction [26]. The ability of the plant extracts to reduce ferric ions was depicted in Table 2.In aqueous extract (0.050 µm AAE/ gw) having more ferric ion reducing activities as compared to methanolic (0.026 µm AAE/ gw) and ethanolic extracts (0.027 µm AAE/ gw).

Ferrous ion chelating activity

Ferrous ion, commonly found in food systems, is well known as an effective pro-oxidant [27]. The ferrous ion chelating activity of the fruit extracts are shown in Table 2.Ethanolic extracts (61.75%) showed highest ferrous ion chelating activity followed by methanolic (59.62%) and aqueous extracts (59.94%).

Table 2: Antioxidant capacities of fruit extracts obtained from different solvents extraction systems

Solvents	DPPH Inhibition (%)	FRAP (µm AAE/ gw)	Ferrous ion chelating activity (%)
Aqueous	1.356 ± 0.06	0.050 ± 0.02	59.94±0.013
Methanol	2.256 ± 0.14	0.026 ± 0.12	59.62±0.09
Ethanol	3.872 ± 0.13	0.027 ± 0.01	61.75±0.012

Values expressed are means ± S.D. of three replicates

Volatile compounds

Phytocomponents in methanolic, ethanolic and petroleum ether extracts of *Aegle marmelos* fruit by GC-MS Report

Volatile compounds in methanolic extract of bael fruit by GC-MS

The compounds present in the methanolic fruit extract of bael fruit were identified by GC-MS

analysis presented in figure 1.The active principles with their retention time (RT), molecular weight (MW) and concentration (%) are presented in Table 3. Twelve compounds were identified in the extract being Quinoline (19.09 %), Hexacosane (16.43 %), Nanocosane (14.79 %), Tetracontane (11.13 %), Pranqenin (10.63 %), Tetracosane (9.56 %), Azulene (6.27 %), 1,3Cyclohexane (5.78 %), Carophyllene (2.27 %), Gamma Elemene (1.59 %), Beta Sesquiphellandren (1.32 %), Cyclohexane (1.12 %).

Table 3: Volatile compounds in methanolic extract of bael fruit by GC-MS

Peak#	R Time	I Time	F Time	Area	Area %	Name of Compound
1	10.982	10.933	11.017	5108078	1.12	Cyclohexane
2	11.407	11.367	11.442	10317671	2.27	Carophyllene
3	11.506	11.467	11.533	7224266	1.59	Gamma Elemene
4	11.753	11.725	11.783	6016869	1.32	Beta Sesquiphellandren
5	12.23	12.2	12.267	26304177	5.78	1,3 Cyclohexane
6	13.094	13.058	13.142	28525276	6.27	Azulene
7	20.651	20.517	20.783	86827954	19.09	Quinoline
8	21.661	21.583	21.775	48328768	10.63	Pranqenin
9	22.689	22.6	22.85	43479113	9.56	Tetracosane
10	23.734	23.633	23.858	74743017	16.43	Hexacosane
11	24.339	24.217	24.433	50632631	11.13	Tetracontane
12	24.693	24.608	24.775	67281728	14.79	Nanocosane

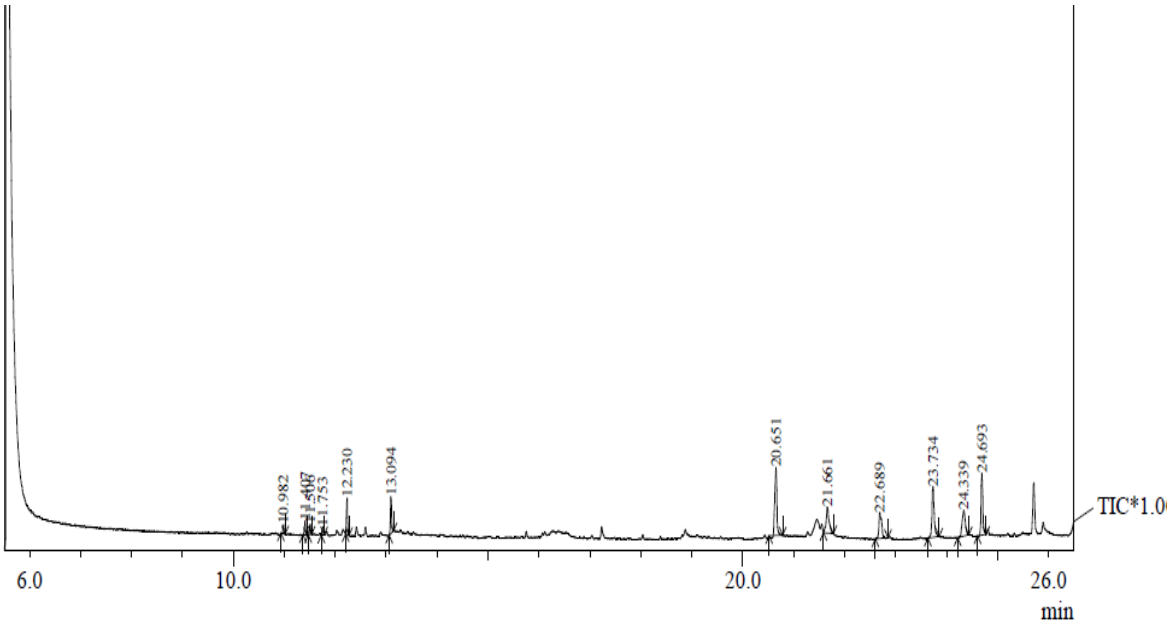

Fig 1: GC-MS spectrum of methanol extract of bael fruit

Volatile compounds in ethanolic extract of bael fruit by GC-MS Report

The compounds present in the methanolic fruit extract of bael fruit were identified by GC-MS analysis presented in figure 2. The active principles with their retention time (RT), molecular weight (MW) and concentration (%) are presented in Table 4. Ten compounds were identified in the extract being Nanocosane (19.42 %), 4-Hydroxy-7H Furo(3-2-g) Chromen-7-One (15.43 %), Tetracosane (11.16 %), Prangenin (10.09 %), Hexacosane (9.05 %),Tetracosane (8.18 %), Tetracontane (8.18 %), Gamma- Elemene (7.34 %), 1, 3 Cyclohexadiene (7.25 %), Squalene (3.36 %).

Table 4: Volatile compounds in ethanol extract of bael fruit by GC-MS

Peak#	R Time	I Time	F Time	Area	Area %	Name of Compound
1	12.226	12.2	12.292	3E+07	7.25	1,3 Cyclohexadiene
2	13.089	13.033	13.175	3E+07	7.34	Gamma- Elemene
3	20.64	20.55	20.725	6.3E+07	15.43	4-Hydroxy-7H Furo(3-2-g)Chromen-7-One
4	21.661	21.558	21.783	4.1E+07	10.09	Prangenin
5	22.723	22.608	22.825	3.4E+07	8.18	Tetracosane
6	23.748	23.608	23.908	8E+07	19.42	Nanocosane
7	24.325	24.208	24.45	3.6E+07	8.73	Tetratetracontane
8	24.704	24.6	24.792	4.6E+07	11.16	Tetracosane
9	25.718	25.625	25.808	3.7E+07	9.05	Hexacosane
10	25.899	25.833	25.983	1.4E+07	3.36	Squalene

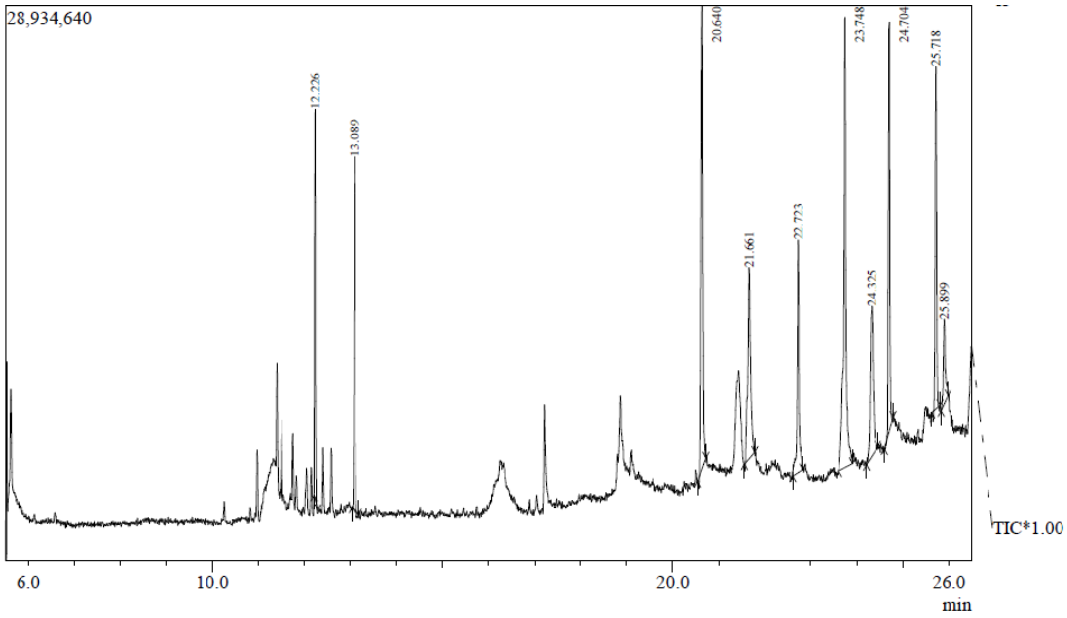

Fig 2: GC-MS spectrum of ethanol extract of bael fruit

Volatile compounds in petroleum ether extract of bael fruit by GC-MS

The compounds present in the petroleum ether fruit extract of bael fruit were identified by GC-MS analysis presented in figure 3.The active principles with their retention time (RT), molecular weight (MW) and concentration (%) are presented in Table 5. Twenty one compounds were identified in the extract being Nanocosane (18.12 %), Hexocosane (15.47 %), Nanocosane (13.87 %), Tetracosane (9.22 %),4-Hydroxy-7H- Furo(3-2-g)Chromen-7-One (8.11 %), 1, 3 Cyclohexidiene (7.01 %), Azulene (6.53 %), Squalene (3.04 %), Tetratetracontane (3.00 %), Caryophyllene (2.18 %), n-Hexadecanoic acid (2.07 %), Gamma-Elemene (1.55 %), Heneicosane (1.51 %), Docosane (1.33 %),Cyclohexane (1.27 %), 3-(1,5-dimethyl 4hexenyl)-6-methylene (1.25 %), 1-methyl-4-(5-methyl1methylene-4-hexenyl) (1.12 %), Cyclohexane (0.97 %), Germacrene (0.85 %), Octadecane (0.74 %) and Hexadecane (0.44 %).

Table 5: Volatile compounds in petroleum ether extract of bael fruit by GC-MS

Peak#	R Time	I Time	F Time	Area	Area %	Name of Compound
1	10.978	10.925	11.025	7689794	1.27	Cyclohexane
2	11.404	11.35	11.458	1.3E+07	2.18	Caryophyllene
3	11.502	11.467	11.558	9389182	1.55	Gamma-Elemene
4	11.75	11.7	11.783	5864232	0.97	Cyclohexane
5	12.146	12.108	12.192	5144846	0.85	Germacrene
6	12.227	12.183	12.283	4.2E+07	7.01	1,3 Cyclohexidiene
7	12.41	12.367	12.467	6793950	1.12	1-methyl-4-(5-methyl1methylene-4-hexenyl)
8	12.587	12.558	12.642	7581092	1.25	3-(1,5-dimethyl 4hexenyl)-6-methylene
9	13.092	13.042	13.142	4E+07	6.53	Azulene
10	13.539	13.508	13.567	2685972	0.44	Hexadecane
11	17.231	17.175	17.325	1.3E+07	2.07	n-Hexadecanoic acid
12	17.747	17.683	17.792	4470134	0.74	Octadecane
13	19.57	19.508	19.625	8078300	1.33	Docosane
14	20.517	20.475	20.558	9154218	1.51	Heneicosane
15	20.638	20.583	20.725	4.9E+07	8.11	4-Hydroxy-7H- Furo(3-2-g)Chromen-7-One
16	21.617	21.55	21.658	1.8E+07	3	Tetratetracontane
17	22.726	22.633	22.8	5.6E+07	9.22	Tetracosane
18	23.752	23.592	23.858	1.1E+08	18.12	Nanocosane
19	24.707	24.642	24.808	9.4E+07	15.47	Hexocosane
20	25.724	25.65	25.792	8.4E+07	13.87	Nanocosane
21	25.904	25.85	25.967	2.1E+07	3.4	Squalene

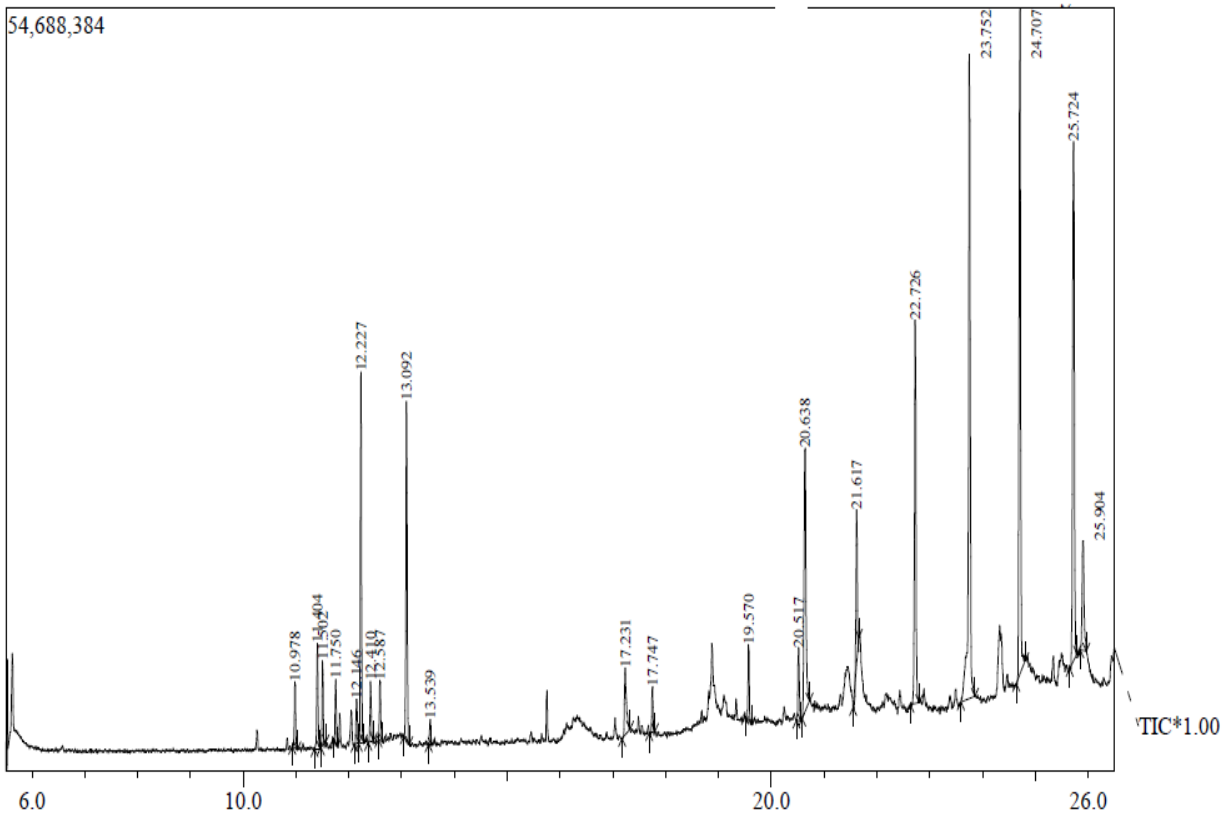

Fig 3: GC-MS spectrum of petroleum ether extract of bael fruit

4. CONCLUSION

Based on the result in the study, it was concluded that extracts of *Aegle marmelos* fruit pulp were found to be a good natural antioxidant. Further studies are required to identify specific active principles of this plant for the significant antioxidant effect. From the present study, it is concluded that the maximum extraction of phytochemicals was observed in petroleum ether extract than methanolic and ethanolic extract which reveals that Nanocosane is highly valuable in medicinal usage for the treatment of various human aliments.

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