International Research Journal of Natural and Applied Sciences
Vol. 4, Issue 2, February 2017 Impact Factor- 5.46
ISSN: (2349-4077)

Website: www.aarf.asia Email : editor@aarf.asia , editoraarf@gmail.com

GC-MS ANALYSIS OF *AEGLE MARMELOS* CORREA EX ROXB. (RUTACEAE) LEAF METHANOL EXTRACT

Dr. Shubhangi N. Ingole and Rupali S. Kaikade
Dept of Botany, Bar. RDIK and NKD College, Badnera, Dist Amravati. Maharashtra, India.

ABSTRACT

Plants have been an important source of medicine with qualities for thousands of years. Mainly on traditional remedies such as herbs for their history it has been used as a popular folk medicine. Aegle marmelos has medicinal values and hence methanol leaf extract of this plant was analyzed using Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the Central Instrumentation library, Punjab University, Chandigarh. Gas chromatography mass spectrometry (GC-MS) analysis revealed the presence of 15 compounds. In GC-MS analysis, some of the phytocomponents screened were Phytol, Squalene and sitosterol. The compounds were identified by comparing their retention time and peak area with that of literature and by interpretation of mass spectra. Many of them are used in industry for various applications like flavor, antioxidant, anti-inflammatory, antimicrobial, pesticide and cancer preventive.

Keywords: *Aegle marmelos*, GC-MS, Phytol, Squalene.

World is endowed with a rich wealth of medicinal plants and India is sitting on a gold mine of well recorded and traditionally well- practiced knowledge of herbal medicine. Herbal medicine is defined as a plant derived material or preparations which contain raw or processed ingredients from one or more plants with therapeutic value (WHO, 1993). More than 1500 herbal preparations are sold as dietary supplements or ethnic traditional medicines (WHO, 2000). Our country is perhaps the largest producer of medicinal herbs and is rightly called the botanical garden of the world (Parrotta, 2001and Agrawal *et al*., 2005).The herbal drugs provide alternative and effective treatment for chronic disorders (Astin, 1998 and Cupp, 1999) and various diseases predates human history from the origin of much of the

modern medicines. They also offer therapeutics for age related disorders like memory loss, osteoporosis and immune disorders for which no modern medicine is available.

Man cannot survive on this earth for long time without the plant kingdom because the plant products and their active constituents play an important role. There is a wide spread belief that green medicines are healthier and more harmless or safer than synthetic ones. Because active constituents in plants are always biologically balanced, affect the human being in complex manner, do not accumulate in the body and are even capable of neutralizing the harmful effects of other, usually chemical compounds. Phytochemical is a natural bioactive compound found in plants such as vegetables, fruits, medicinal plants, flowers, leaves and root that work with and fibers to act as a defense system against diseases of more accurately, to protect against diseases (Krishnaiah *et al*., 2009).

Aegle marmelos (L.) Corr. belonging to family Rutaceae. All parts of *Aegle marmelos* are medicinally useful like, leaves, fruit pulp, and flower, stem bark, root bark. Ripe fruit is sweet, aromatic, cooling, alterative, and nutritive. When taken fresh, it is useful in constipation, chronic dysentery and dyspepsia. Unripe fruit is astringent, digestive, stomachic and demulcent. Pulp is stimulant and antipyretic. Fresh juice is bitter and pungent. Root and stem bark are used as antipyretic.

The aim of the present study is to identify the phytocomponents of this plant and subjecting the methanol extract of the plant leaves to Gas chromatography – Mass Spectrum analysis. In the present study, volatile organic matter of the leaf sample of plant was analyzed for the first time. This work will help to identify the compounds, which may be used in body products or of therapeutic value. Mass spectrometry is the most sensitive and selective method for molecular analysis and can yield information on the molecular weight as well as the structure of the molecule. Combining chromatography with mass spectrometry (GC-MS) provides the advantage of both chromatography as a separation method and mass spectrometry as an identification method. In mass spectrometry, there is a range of methods to ionize compounds and then separate the ions.

MATERIALS AND METHODS

After the proper identification, bark and leaves were cut it into small pieces and shade dried at room temperature and made into coarsely powdered using mechanical grinder and preserved in air tight container. For the phytochemical sreening, the method of Chhabra (1984) was adopted.

Preparation of extract

15g powder of plant material was soaked in 150ml of petroleum ether. It was kept for 24 hrs. After 24 hrs, the material extracted with ether and named as 'Ether extract. Then petroleum ether was distilled off and repeatedly extracted with methanol (150ml) and named as 'Methanol extract.

GC-MS Analysis

The GCMS analysis was conducted at the Central Instrumentation Laboratory, Punjab University at Chandigarh. 2µL aliquot was injected into a fisons GC8000 series coupled to a TSQ8000 MS (Triplequadrapole) mass analyzer .The chromatography was performed by using the DB5-MS column. Injection temperature was 230°C. Helium flow was 1mL/min. After a 5 min solvent delay time at 70°C; the oven temperature was increased at 5°C/min to 310°C, 1min isocratic and cooled to 70°C, followed by the additional 5min delay. The ion trace integration was done using the mass lab find target method for the characteristic fragment of assigned peaks.

Identification of Components

Interpretation of mass spectrum GCMS was conducted using data base of the Central Instrumentation Laboratory (CIL) spectra Libraries, Punjab University, Chandigarh. Spectrum of the unknown component was compared with the spectrum of known components stored in the CIL. The molecular weight, molecular formula and the number of hits used to identify the name of the compound from CIL spectra Libraries were recorded.

This is done in order to determine whether this plant species contains any individual compound or group of compounds, which may substantiate its current commercial and traditional use as an herbal medicine. Further it helps to determine the most appropriate methods of extracting these compounds. These results will consequently be discussed in the light of their putative biological or therapeutic relevance.

RESULTS

The phytochemical profile of different plant parts in different extracts is given in the following table.

Plant part	Alkaloids			Anthracene Glycosides		Anthroquinone	Aucubins	Iridoids	Carotenoids
	E	M	W	M	W				E
Stem bark	+	+	-	+	+	+	+	-	-
Leaves	+	+	+	+	+	-	-	-	-

Plant part	Coumarin			Emodin	Fatty Acid	Flavonoid			Polyuronoid	Tannin		Starch
	E	M	W	E	E	E	M	W	W	M	W	W
Stem Bark	-	-	-	-	-	-	-	-	-	+	+	-
Leaves	-	-	-	-	-	-	-	-	-	+	-	-
Flower	-	-	-	-	+	+	+	+	-	-	-	+

Plant part	Polyoses	Reducing Compounds		Saponin	Steroids			Triterpenoids			Volatile Oil
	W	M	W	W	E	M	W	E	M	W	E
Stem Bark	-	-	-	+	-	-	-	+	+	+	-
Leaves	-	+	+	+	+	+	+	-	-	-	+
Flower	+	-	-	-	-	-	-	+	+	+	+

Table 1: Phytochemical profile of different plant parts in different extracts.

GC-MS is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids, esters etc. The GC-MS analysis of A. marmelos leaves revealed the presence of seventeen compounds (phytochemical constituents) that could contribute the medicinal quality of the plant. The identification of the phytochemical compounds was confirmed based on the peak area, retention time and

molecular formula. The active principles with their Retention time (RT) and Molecular formula are presented in Table 2 and Figure 1.

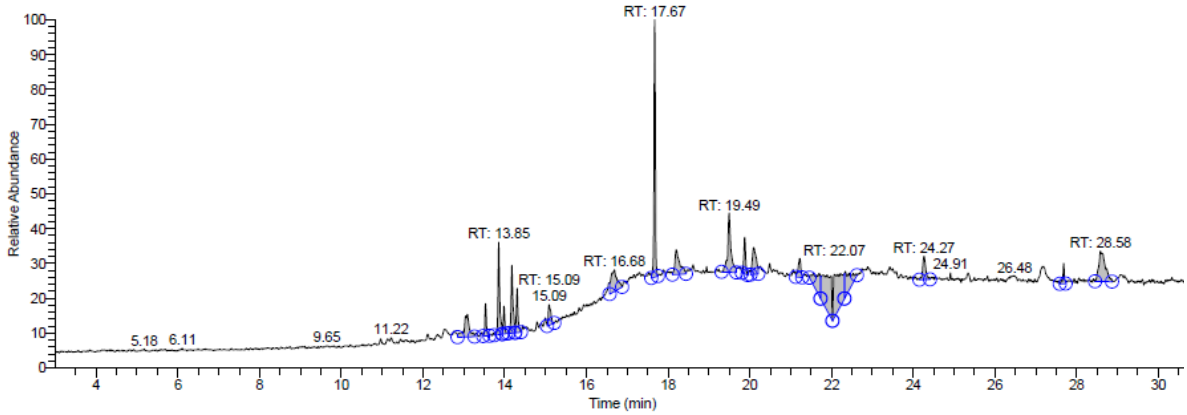

Figure 1: Spectra of Gas Chromatography of leaves of *A. marmelos* in methanol extract.

S.N.	Retention Time	Components	Molecular Formula
1	13.09	13- Heptadecyn-1-ol	$C_{17}H_{32}O$
2	13.53	Dibutyl phthalate	$C_{16}H_{22}O_4$
3	13.85	1,2- Benzenedicarboxylic acid,	$C_{16}H_{22}O_4$
4	13.98	Phthalic acid	$C_{18}H_{26}O_4$
5	15.09	Phytol	$C_{20}H_{40}O$
6	16.68	Ethyl iso- allocholate	$C_{26}H_{44}O_5$
7	17.67	Diisooctyl phthalate	$C_{24}H_{38}O_4$
8	18.20	Vinyl phenyl acetonitrile	$C_{10}H_9N$
9	19.49	13- Docosenamide	$C_{22}H_{43}NO$
10	19.87	Squalene	$C_{30}H_{50}$
11	20.09	Pentoic acid	$C_{13}H_{18}O_4$
12	21.22	Oxirane	$C_{30}H_{50}O$
13	24.27	Vitamin E, á- Tocopherol	$C_{29}H_{5o}O_4$, $C_{29}H_{5o}O_2$
14	27.68	Carotene, 9,12,15- Octadecatrienoic acid	$C_{42}H_{64}O_2$, $C_{42}H_{64}O_2$,
15	28.58	ç- Sitosterol	$C_{29}H_{50}O$

Table 2: GC-MS analysis revealed the presence of phytochemical components in methanol leaf extract of *A. marmelos*.

The phytochemicals identified through GC-MS analysis possess many biological properties. For instance, 9,12,15-Octadecatrienoic acid possesses anti-inflammatory, insectifuge, hypocholesterolemic, cancer preventive, nematicide, hepatoprotective, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic and anticoronary properties (Sermakkani *et al*., 2012).

1,2- benzenedicarboxylic acid and diisooctyl phthalate are known to possess antimicrobial and antifouling activity. Phytol is an antimicrobial, anticancer, anti-inflammatory and diuretic agent (Praveen kumar *et al*., 2010). Phytol was observed to have antibacterial activities against *Staphylococcous aureus* by causing damage to cell membranes as a result there is a leakage of potassium ions from bacterial cells. Phytol is a key acyclic diterpene alcohol that is a precursor for vitamins E and K1. It is used along with simple sugar or corn syrup as a hardener in candies.

Dibutyl phthalate possess antimicrobial and antifouling properties. Ethyl iso-allocholate is reported to possess antimicrobial, diuretic, anti-inflammatory properties.

Squalene is a naturally occurring polyprenyl compound primarily known for its key role as an intermediate in cholesterol synthesis. It receives its name because of its occurrence in shark liver oil (Squalus species) which contains large quantities and considered the richest source of squalene. Squalene is a natural antioxidant, aunique oxygen generator, power immune stimulator, antibiotic, anti-coagulant, anti-histamine and anti-allergic (Kelly, 1999). It has been proposed to be an important part of the Mediterranean diet as it may be a chemo preventative substance that protects people from cancer (Smith and Theresa, 2000 and Owen, 2004).

Vitamin E has many biological functions; the antioxidant function being the most important and best known. Vitamin E also plays a role in neurological functions. Vitamin E also protects lipids and prevents the oxidation of polyunsaturated fatty acids (PUFAs). So far, most human supplementation studies about vitamin E have used only alpha-tocopherol. This can affect levels of other forms of vitamin E, e.g. reducing serum gamma- and delta-tocopherol concentrations.

DISCUSSION AND CONCLUSION

The more precise information in qualitative analysis can be obtained by gas-chromatography coupled with mass spectrometry (GC-MS). The GC-MS analysis of *A. marmelos* leaves revealed the presence of fifteen compounds. The identified compounds possess many biological properties.

The source of many plants (herbs and spices) can often be identified from the peak pattern of the chromatograms obtained directly from headspace analysis. Similarly, unique qualitative and quantitative patterns from a GC analysis will often help identify the source of many alcoholic beverages. The fundamental reason of quality control of herbal medicines is based on the concept of phytoequivalence of herbs, and then to use this conception to identify the real herbal medicine and the false one, and further to do quality control.

Therefore, GC-MS method is a direct and fast analytical approach for identification of terpenoids and steroids and only few grams of plant material is required. The importance of the study is due to the biological activity of some of these compounds. The present study, which reveals the presence of components in *A. marmelos* suggest that the contribution of these compounds on the pharmacological activity should be evaluated.

REFERENCES

1. Astin, J. (1998). Why patients use alternative medicines: Results of national study. J Am Med Assoc, 279: 1548- 1553.
2. Agrawal, S.S.; Tambekar, B.P. and Paridhavi, M.B. (2005). Clinically useful herbal drugs. 1st Edn, Ahuja Publishing House, Delhi.
3. Chhabra, S.C.; Ulso, F.E and Mshiu, E.N. (1984). Phytochemical Screening of Tanzanian Medicinal Plant- I. Journal of Ethnopharmacology, 11:157- 79.
4. Cupp, M. (1999). Herbal remedies: Adverse effects and drug interaction. Am Fam Physician, 59: 1239-1244.
5. Kelly, G.S. (1999). Squalene and its potential clinical uses. Altern Med Rev, 4(1):29-36
6. Krishnaiah D, Devi T, Bono A and Sarbatly R: Studies on phytochemical constituents of six Malaysian medicinal plants. *J. Med. Plant Res* 2009; 3(2): 67-72.
7. Owen, R. W.; Haubner, R.; Würtele, G.; Hull, W E.; Spiegelhalder, B. and Bartsch, H. (2004). Olives and olive oil in cancer prevention. European Journal of Cancer Prevention, 13(4): 319-326.
8. Parrotta A.J. (2001). Healing plants of Peninsular India, CABI Pub., New York, 308.

9. Praveen kumar, P.; Kumaravel, S. and Lalitha, C. (2010). Screening of antioxidant activity, total phenolics and GC-MS study of Vitex negundo. Afr. J. Biochemistry Res, 4 (7): 191-195.
10. Sermakkani. M. and Thangapandian (2012). GC-MS Analysis of Cassia italic leaf methanol extract. Asian Journal of pharmaceutical and clinical Research, 5(2) -90-94.
11. Smith and Theresa, J. (2000). Squalene: potential chemopreventive agent. Expert Opinion on Investigational Drugs, 9(8): 1841–1848.
12. WHO (1993). Summary of WHO guidelines for the assessment of herbal medicines. Herbal Gram, 28: 13-14
13. WHO (2000). General gulidelines for methodology on research and evaluation of traditional medicines, World Health Organisation, Geneva.