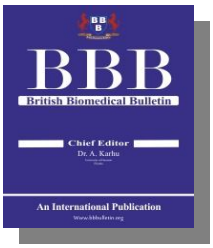
Journal Home Page www.bbbulletin.org

BRITISH BIOMEDICAL BULLETIN



Original

GC–MS analysis of ethyl acetate extract of *Phyllanthus emblica* L. bark

P. Deepak* and G.V. Gopal

Regional Institute of Education, University of Mysore, Mysore, Karnataka, India

ARTICLE INFO

Received 11 Mar. 2014
Received in revised form 04 Mar. 2014
Accepted 11 Apr. 2014

Keywords:
Ethnomedicine, Kurumba, Kundah, Nilgiri.

Corresponding author: Research Scholar, Regional Institute of Education, Mysore – 560 007
E-mail address:
deepakpuravankara@gmail.com

ABSTRACT

Phyllanthus emblica posses a vast ethnomedicinal history and represents a large group of phytochemical reservoir of medicinal uses. It is one of the ingredients used from time immemorial in various ancient literatures like in “Ayurveda” and “Charka Samhitha” as a potential ingredient for various ailments. The fruit is studied for various phyto chemical constituents like rich in quercetin, gallic acid, tannins, flavonoids, pectin and Vitamin C and also contains various polyphenolic compounds. A wide range of phytochemical components from this plant have shown proven results for biological activities like terpenoids, alkaloids, flavonoids and tannins. Many pharmacological studies also have exhibited proven results for antioxidant, anticarcinogenic, antitumour, antigenotoxic, antiinflamatory activities supporting its traditional uses. In view of these studies the present work has been focused on the GC–MS analysis of ethyl acetate extract of the bark portion of the *P. emblica* which could be a possible source of extracting the therapeutically useful products.

Introduction

Amla (*Phyllanthus emblica* L.) is a precious gift of nature to the entire mankind. It also forms an integral part of our various traditional medicinal practises like Ayurveda, Siddha and Unani systems of medicine with wonderful healing qualities. In Sanskrit the plant is called as Amalaki or Dharttiphala. In India the fruit is been widely used for various medicinal preparations like the fruit which is used as a major ingredient in most of the ayurvedic recipe like chyvanaprasha, churnas and pills, similarly it is also used as a part of the regular food like pickles and sausages. The fruit contains 80% of water along with protein, carbohydrate, fiber, minerals and gallic acid. It is also a rich source of Vitamin C (ascorbic acid) which very essential for

human beings. Since vitamin C is a water-soluble vitamin and is very essential for human diet because the body is unable to synthesise it, hence it is naturally supplied through various natural products like fruits and vegetables. Vitamin C is also essential for collagen formation and helps to maintain the integrity of skin and connective tissue, bone, blood vessel walls and dentine, severe deficiency of vitamin C results in scurvy, which is characterised by haemorrhages, abnormal bone and dentine formation. It is also essential for wound healing and facilitates recovery from burns. It also been used in the absorption of iron. Vitamin C is a good antioxidant[1].

Morphological characters of the plant sample

The tree is small medium sized, reaching a height of up to 6-8 m with a crooked trunk and spreading branches. The branch lets are glabrous, 10 -20 cm long, deciduous. The leaves are simple, sub sessile and closely set along the branchlets. The flowers are greenish – yellow. The fruit is small, spherical, greenish – yellow in colour. The taste is sour, bitter and astringent and fibrous[2].

Phytochemical Constituents

The major biologically active phytochemical constituents are terpenoids, alkaloids, flavonoids and tannins[3, 4]. The fruits, leaves and bark are rich in tannins. The roots contain ellagic acid and lupeol. The seeds yield a fixed oil (16%) which is brownish- yellow in colour. The plant contains following fatty acids: linolenic, oleic, stearic, palmitic, linoleic and myristic[5]. The hydrolysable tannins Emblicanin A, Emblicanin B, punigluconin, pedunculagin[6], Flavonoids like (Kaempferol 3 O alpha L (6" methyl) rhamnopyranoside, Kaempferol 3 O alpha L (6" ethyl) amnopyranoside)[7], alkaloids like phyllantidine and phyllantine[8]. Gallic acids, ellagic acid, 1- Ogalloyl – beta – D-glucose, chebulinic acid, quercetin, chebulagic acid and corilagin together with isostrictinnin were isolated from the fruit of *P. emblica*[9]. Ellagic acid and lupeol are present in the roots of *P. emblica*[10, 11]. From the literature review it's clear that *P. emblica* is a wonder herb and is a nature's boon to mankind to cure various ailments. Hence the present study was designed to understand the phytochemical constituents of the bark part of the plant which was widely used in the Kurumba pharmacopeia by keeping in mind the medicinal properties of the fruit and leaves which was reported by earlier workers.

Material and Methods

The plant samples were collected as a part of the ethnobotanical exploration which was carried out in the Kurumba settlement called Belathicombai in Onikandi near Manjor town in Kundah taluk during 2009-2010(Figure 1and 2). All the collected plant specimens were identified taxonomically with the help of The Flora of Presidency of Madras[12], The Flora of Tamil Nadu Carnatic[13] and The Flora of South Indian Hill Station[14]. Herbarium was prepared by following the procedure described in Methods and Approaches in Ethnobotany[15]. The voucher specimens were deposited at the RIEM herbarium.

Preparation of the bark extract

Fresh plant materials (tender bark) of *Phyllanthus emblica* which are free from diseases were collected from the study area. The bark was washed thoroughly 2-3 times with running water and one's with sterile distilled water. The material was then shade dried on a sterile blotter for 40 days, afterwards in a ventilated oven for 40^0C and subsequently milled to a fine powder by means of a blender and sieved. The required

quantity of the sample was weighed and transferred to a Stoppard flask and then treated with ethyl acetate until the powder was fully immersed and shaken, then incubated overnight. The extract was then filtered using Whatmann filter paper No. 41. The extract collected and evapourated to dryness by using a vaccum distillation unit and the final residue was used for GC-MS analysis.

GC–MS analysis

1µl of the ethyl acetate extract of *Phyllanthus emblica* was used for the carrying out the GC-MS analysis for various phytochemical compounds.

GC- MS analysis was carried out using a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25mm × ID × 1 µm of capillary column, composed of 100% Dimethyl poly siloxane), operating in electron ionization system with an impact mode of 70 eV was used ; helium (99.998%) was used as carrier gas at a constant flow rate of 1ml/min and an injection volume of 1 µl was employed split less, injector temperature of 250^0C; ion – source temperature 280^0C. The oven temperature was programmed from 40^0C (isothermal for 5 min), with an increase of 10^0C/min, to 300^0C/min isothermal; then hold for 5 mins. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450Da. Total GC run time was 34 mins.

Identification of the phytochemical constituents

The interpretation of the mass spectrum GC-MS was carried out using the database of National Institute Standards and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of the sample were ascertained using NIST Ver. 2.1 MS data library.

Result and Discussion

The study on the active principles of ethyl acetate extract of the stem bark of *Phyllanthus emblica* using GC- MS showed the presence of 21 major and minor peaks. The GC- MS chromatogram of the twenty one peaks of the compounds detected are shown in the figure – 3. The major and minor compounds with their retention time (RT), molecular formulae, molecular weight (MW) and peak area (%) are presented in the Table: 1. The compound nature of the five major phytochemical constituents include terpenes, terpenoids and steroids as depicted in figure – 2. like Lupeol (8.54%) and Betulin (0.54%) falls under the terpene category whereas Friedelan – 3- one (2.30%) is a terpenoid , β – Humulene (11.82%) is a sesquiterpene and Stigmasterol (0.48%) which is a phytosterol. Most of these compounds have shown proven medicinal values in the pharmaceutical industries like Lupeol which exhibits the anti-inflammatory, antiperoxidant, anti tumour and anti rheumatic properties[16]. Betulin can be easily converted to betulinic acid, which possesses a wide spectrum of biological and pharmacological activities like anti HIV, anti carcinogenic, anti flu, anti inflammatory, anti tumour, anti viral[17].Thus the bark part of the plant also forms a new source for the extraction of these phytocompounds. Stigmasterol has shown proven results for anti-hypercholesterolemic studies[18]. Studies have also revealed the antiulcerogenic activity of Friedelan– 3- one from *Maytenus ilicifolia*[19].

Conclusion

The GC- MS analysis of bark extract *Phyllanthus emblica* clearly exhibits the presence of terpenes, phytosterols and terpenoids which are mainly present in the plants for protecting them from the deterring parasites. These chemical constituents play a major role for the efficacy of these traditional herbal remedies. Some of the therapuetical values of the major phytochemical constituents are provided in Table .2. The study also pave way for further possibility of extraction of these important phyto compounds from the bark part of the plant which was not earlier reported. The studies also indicated the presence of lot of other phyto compounds which are not tested for their biological activity. Hence further studies are needed to be worked out on the application of these phytochemical compounds in drug delivery and pharmaceutical fields.

Author's Contribution and Competing Interests

Acknowledgement

The authors express their gratitude to the Central facility Biochemistry, IISc., (Indian Institute of Sciences), Bangalore for providing the laboratory facility and also to the Kurumba healers for sharing their medicinal practices.

References

1. Singh E, Sharma S, Pareek A, Diwedi J, Yadav S and Sharma S, "Phytochemistry, traditional uses and cancer chemopreventive activity of Amla (*Phyllanthus emblica*): The sustainer." *J. App. Pharma. Sci.* 2011; 02(01):176-183.
2. Brun V, Schumacher T, "Traditional Herbal medicine in Northern Thailand." *University of California Press, Berkley*, 1987, 349 -358.
3. Kim HJ, Yokozawat, Kimhy, Tohda C, Rao TP, Juneja LR, "Influence of Amla (Emblica officnalis Gaerntl) on hypercholesterolemia and lipid peroxidation in cholesterol – fed rats." *J. Nutr. Sci. Vitaminol.* 2005; (51): 413-418.
4. Arora S, Kaur K, Kaur S, "Indian medicinal plants as a reservoir of protective phytochemicals." *Teratog Carcinog Mutagen*, 2003; (1): 295-300.
5. Thakur RS, Puri HS, Hussain, Akhtar, "Major medicinal Plants of India." *Central Institute of Medicnal and Aromatic Plants*, Lucknow, India, 1989.
6. Ghosal S, Tripathi VK, Chauhan S, "Active constituent of *Emblica offcinalis*: part 1st the chemistry and antioxidant effects of two new hydrolysable tannins, emblicanin A and B." *Indian J Chem.* 1996; (35b): 941-948.
7. Rahman I, Dutta BK, Das TK, "Antifungal activity of Indian plant extracts." *Mycoses*, 1998; 41(11-12): 535-536.
8. Khanna P, Bansal R, "Phyllantidine and phyllantine from *Emblica officinallis Gaertn.* leaves, fruit and in vitro tissue cultures." *Indian J. Exp. Biol.* 1975; (13):82 -83.
9. Zhang LZ, Zhao WH, Guo YJ, Tu GZ, Lin S, Xin LG, "Studies on chemical constituents in fruits of Tibetan medicine *Phyllanthus emblica*." *Zhongguo Zhong Yao Za Zhi*, 2003; (28): 940-943.
10. Kapoor LD, "Handbook of Ayurvedic Medicinal Plants.", *CRC Press, Boca raton*, 1990.
11. Rastogi RP, Mehrotra BN, "Compendium of Indian Medicinal plant." *CDRI, Lucknow and Publication and Information Directorate*, New Delhi, 1993.
12. Gamble JS and Fischer CEC, "The Flora of the Presidency of Madras." Reprinted ed., Vols. I – III, *Botanical Survey of India*, Calcutta, 1959.
13. Mathew KM, "The Flora of the Tamil Nadu Carnatic." *The Rapinet herbarium, St. Joseph's college*, Tiruchirapalli, India, 1983.
14. Fyson PF, "The Flora of the South Indian Hill Station." Vols. I – III, *Govt. Press*, Madras, 1932.
15. Jain SK, "Ethnobotany: An interdisciplinary science for holistic approach to man plant

relationships". *Methods and Approaches in Ethnobotany*, Jodhpur, 1989, 9-12.
16. Gallo MBC and Sarachine MJ, "Biological activities of Lupeol." *Int. J. Biomedical and Pharmaceutical sciences*, 2009; 2(3): 13 – 18.
17. Alakurtti S, Makela T, Koskimies S, Yli – Kauhaluoma J , "Pharmacological properties of the ubiquitous natural product betullin." *European J. Pharmaceutical sci*. 2006; 29(1): 1-13.
18. Chandler RF, Hooper SN, and Ismail HA, "Antihypercholesterolemic studies with sterols: β – sitosterol and stigmasterol." *J. Pharm. Sci*. 1979; (68): 245-247.
19. Queiroga CL, Silva GF, Dias PC, Possenti A and De Caravlho JE, "Evaluation of the antiulcerogenic activity of fridelan - 3β- ol and friedelin isolated from *Maytenus ilicifolia* (Celastraceae)." *J. Ethnopharmacol.* 2000; 72 (3):465-468.
20. Abdullahi SM, Musa AM, Abdullahi MI, Sule MI and Sani YM, "Isolation of Lupeol from the Stem-bark of Lonchocarpus sericeus (Papilionaceae)." *Sch. Acad. J. Biosci*. 2013; 1(1):18-19.
21. Chapman AC. "□-humulene. Isolation of mixture", *J. Chem. Soc*. 1895; 67(54): 780.
22. Marie-France Guidoina, Jin Y, André Pichetteb, Roy C,"Betulin isolation from birch bark by vacuum and atmospheric sublimation. A thermogravimetric study." *Thermochimica Acta*, 2003; (1-2): 153-166.
23. Kamboj A and Saluja AK, "Isolation of stigmasterol and β sitosterol from petroleum ether extract of aerial parts of *Ageratum conyzoides* (ASTERACEAE)." *Int. J. Pharm. and Pharmaceutical Sci.* 2011; 3(1).

Table 1. Phyto components identified from the ethyl acetate extract *Phyllanthus emblica* bark

No	RT	Name of the compound	Molecular formula	MW	Peak Area %
1	12.341	1-Dodecene	$C_{12}H_{24}$	168.31	0.16
2	15.187	1- Tridecene	$C_{13}H_{26}$	182.3455	0.25
3	16.675	Phenol,2,4- bis (1,1-dimethyl ethyl)-	$C_{14}H_{22}O$	206.3239	0.13
4	17.704	Diethyl phthalate	$C_{12}H_{14}O_4$	222.2372	0.89
5	18.978	Heptadecane	$C_{17}H_{36}$	240.4677	0.17
6	19.993	1- Nonadecene	$C_{19}H_{38}$	266.5050	0.28
7	21.358	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.4501	0.16
8	21.708	n – Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241	1.01
9	22.063	1- Octadecene	$C_{18}H_{36}$	252.4784	0.23
10	23.079	9 – Octadecenoic acid (Z) - , methyl ester	$C_{19}H_{36}O_2$	296.4879	0.14
11	23.370	9,12 – Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4455	0.26
12	23.946	1- Docosene	$C_{22}H_{44}$	308.5848	0.13
13	25.395	Lup – 20 (29) – en – 3 - one	$C_{30}H_{18}O$	424.7015	1.51
14	25.395	1- Heneicosanol	$C_{21}H_{44}O$	312.5735	0.71
15	26.663	Lupeol	$C_{30}H_{50}O$	426.7174	8.54
16	26.928	β – Humulene	$C_{15}H_{24}$	204.3511	11.82
17	28.694	9 – Octadecenamide, (Z) -	$C_{17}H_{34}NO$	281.4766	0.84
18	29.528	Octacosane	$C_{28}H_{58}$	394.7601	0.13
19	29.222	Friedelan – 3-one	$C_{30}H_{50}O$	428.7333	2.30
20	30.033	Betulin	$C_{30}H_{50}O_2$	442.7168	0.54
21	33.235	Stigmasterol	$C_{29}H_{48}O$	412.6908	0.48

Table 2. Activity of phyto–compounds identified in the ethyl acetate extracts of the bark of *Phyllanthus emblica* L.

Sl.No.	Name of compound	Compound nature	** Activity
1	Lupeol	Triterpene	Anti-inflammatory, Antiperoxidant, Anti tumour, Anti rheumatic
2	β – Humulene	Sesquiterpene	Anti malarial, Anti plasmodial
3	Friedelan – 3- one	Triterpenoid	Antiulcerogenic
4	Betulin	Triterpenes	Anti HIV, Anti carcinogenic, Anti flu, Anti inflammatory, Anti tumour, Anti viral
5	Stigmasterol	Sterols	Anti hepatotoxic, Anti inflammatory, Antioxidant, Anti viral

***Source: Dr. Duke's phytochemical and Ethnobotanical databases* [online databases].

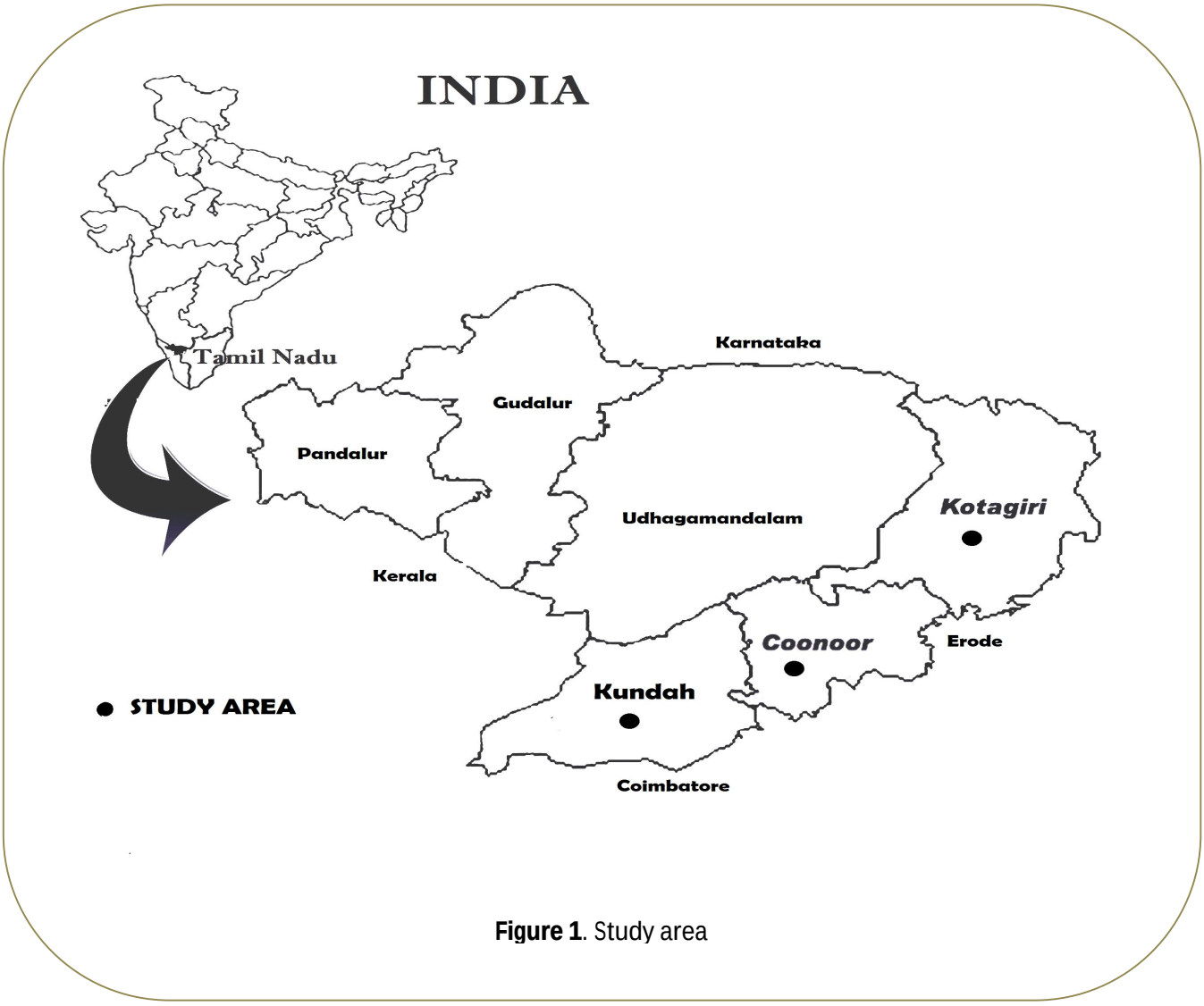


Figure 1. Study area

 ISSN-2347-5447

Figure 2. A. Kurumba traditional healer collecting the bark sample
B. Inflorescence of *Phyllanthus emblica* L.
C. Cut portion of the bark
D. Plant in the habitat.

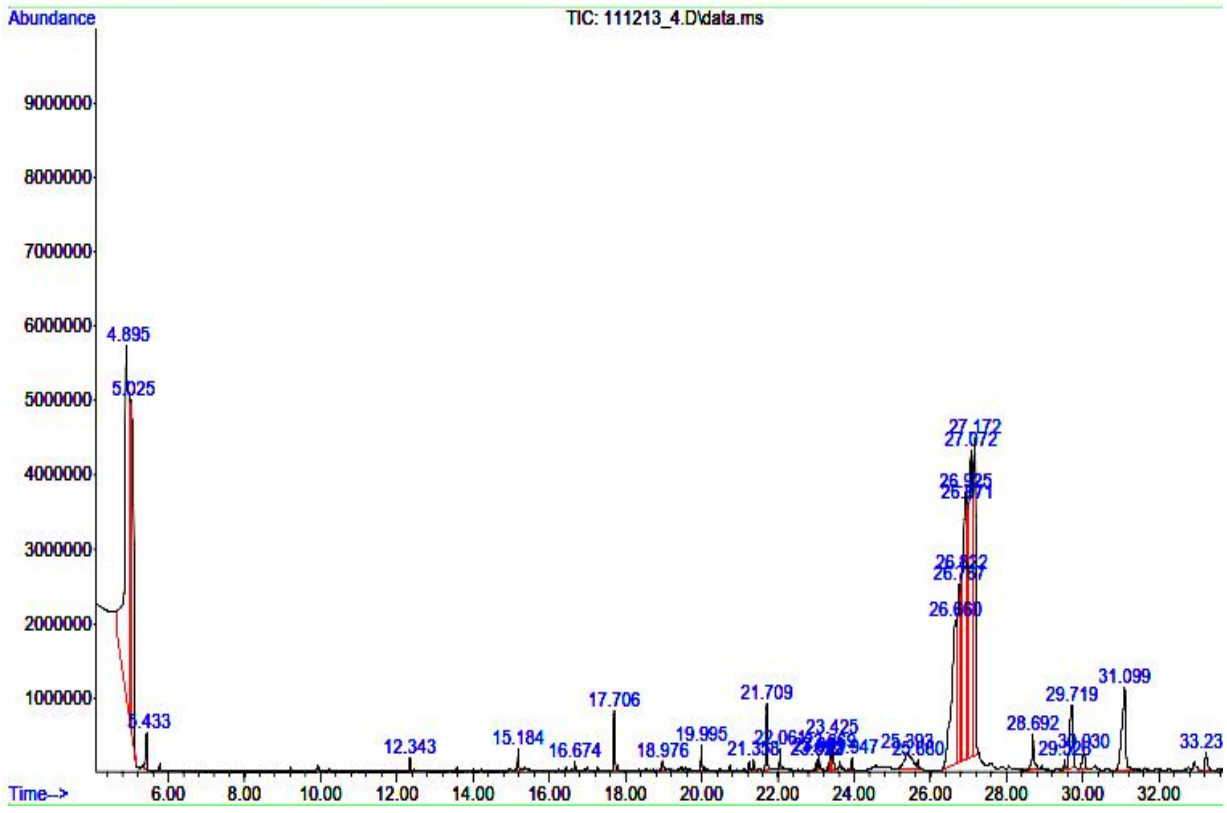
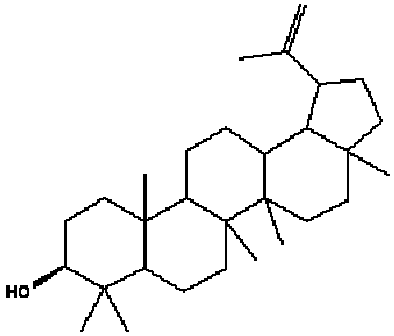
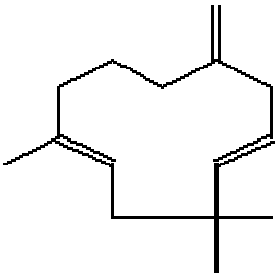


Figure 3. Chromatogram of *Phyllanthus emblica* L. bark by GC-MS

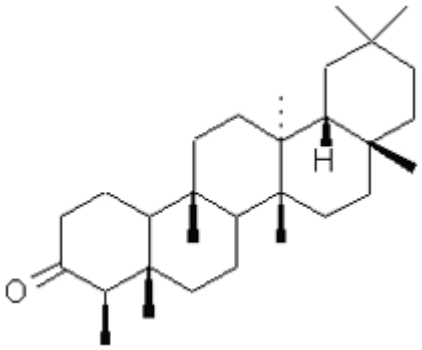
British Biomedical Bulletin



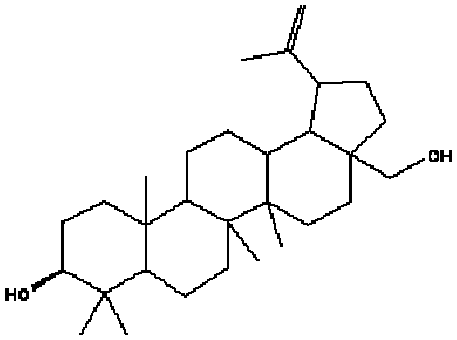
(4.a)



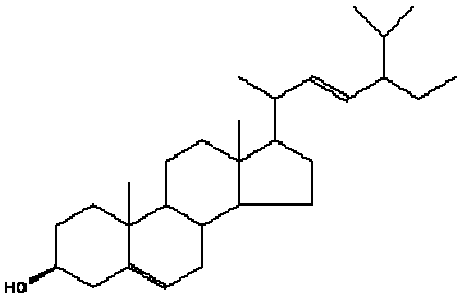
(4.b)



(4.c)



(4.d)



(4.e)

Figure 4. Structural depiction of the major phytocompounds:
4.a: Lupeol, **4.b:** β – Humulene, **4.c:** Friedelan – 3- one, **4.d:** Betulin,
4.e: Stigmasterol