Trad. Med. J., May - August 2016
Vol. 21(2), p 77-85
ISSN : 1410-5918

Submitted : 14-04-2016
Revised : 25-07-2016
Accepted : 05-08-2016

PHYTOCHEMICAL ASSESSMENT ON N-HEXANE EXTRACT AND FRACTIONS OF *Marsilea crenata* Presl. LEAVES THROUGH GC-MS

ANALISIS FITOKIMIA EKSTRAK N-HEKSANA DAN FRAKSI DAUN *Marsilea crenata* Presl. DENGAN GC-MS

Burhan Ma'arif[1*], Mangestuti Agil[1] and Hening Laswati[2]
[1]Department of Pharmacognocy and Phytochemistry, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia
[2]Department of Physical Medicine and Rehabilitation, Faculty of Medicine, Airlangga University, Surabaya, Indonesia

ABSTRACT

Estrogen deficiency causes various health problems in postmenopausal women, including osteoporosis. Phytoestrogen emerged as a potential alternative of estrogen with minimum side effects. Green clover (Marsilea crenata Presl.) is a typical plant in East Java which suspected contains estrogen-like substances. The aim of this research was to report the phytochemical properties of M. crenata using GC-MS as a preliminary study. M. crenata leaves were dried and extracted with n-hexane, then separated using vacuum column chromatography to get four fractions, after that the n-hexane extract and four fractions were identified with GC-MS. The results of GC-MS analysis showed some compounds contained in M. crenata leaves like monoterpenoid, diterpenoid, fatty acid compounds, and other unknown compounds. The results obtained in this research indicated a promising potential of M. crenata as medicinal plants, especially as antiosteoporotic agent.
***Keywords**: Marsilea crenata Presl., phytochemical, antiosteoporosis, GC-MS*

ABSTRAK

Defisiensi estrogen menyebabkan berbagai masalah kesehatan pada wanita pascamenopause, salah satunya osteoporosis. Fitoestrogen muncul sebagai alternatif yang potensial pengganti estrogen dengan efek samping minimal. Semanggi (Marsilea crenata Presl.) merupakan tanaman khas Jawa Timur yang diduga mengandung senyawa dengan fungsi mirip estrogen (estrogen-like substances). Tujuan dari penelitian ini untuk melaporkan kandungan senyawa kimia dalam M. crenata menggunakan GC-MS sebagai studi pendahuluan. Daun M. crenata diekstraksi dengan n-heksana lalu dipisahkan menggunakan kromatografi kolom vakum, setelah itu ekstrak n-heksana dan empat fraksi yang didapatkan diidentifikasi menggunakan GC-MS. Hasil analisis GC-MS menunjukkan beberapa golongan senyawa yang terkandung dalam daun M. crenata seperti monoterpenoid, diterpenoid, senyawa asam lemak, dan senyawa lain yang belum diketahui. Hasil yang diperoleh dalam penelitian ini menunjukkan potensi menjanjikan M. crenata sebagai tanaman obat, khususnya sebagai antiosteoporosis.
***Kata kunci**: Marsilea crenata Presl., fitokimia, antiosteoporosis, GC-MS*

INTRODUCTION

Marsilea. crenata (Figure 1) is a unique plant that grows in East Java, Indonesia. Its leaves are widely used as an ingredient for traditional food (Afriastini, 2003; Nurjanah *et al.* 2012). *M. crenata* is a kind of ferns which is usually grown in the aquatic environment (Afriastini, 2003; Steenis, 1975). Different from other Marsilea plants such as *Marsilea minuta* Linn., *Marsilea quadrifolia*, *Marsilea rajasthansis* Gupta, and *Marsilea drummondii*, the phytochemical properties of *Marsilea crenata* Presl. has not been confirmed yet. Whereas these plants has great potential as a source of food or as medicinal plants and very easy to grow (Yacoeb *et al.* 2010).

Some research shows that *M. crenata* has activity for prevention of osteoporosis (Laswati, 2011). Using radioimmunoassay (RIA), estradiol-like compound concentrations in *M. crenata* leaves were detected quite high (Laswati, 2011), and it has potentials in delaying the increment

Corresponding Author : Burhan Ma'arif
Email : burhan.maarif@gmail.com

of imbalance bone remodeling process in post-menopausal women (Laswati, 2011; Yang, *et al*. 2012; Ososki *et al*. 2003).

Figure 1. *M. crenata*

This study was aimed to report the phytochemical properties of *M. crenata* using GC-MS as a preliminary study to determine the anti-osteoporotic activity of *M. crenata*. GC-MS is a powerful technique used for many applications which has very high sensitivity and specificity and also can save more time (Elezabeth *et al*. 2014; Kumar *et al*. 2014; Bai *et al*. 2014).

METHODOLOGY

Chemical and Equipment

Plant Material

The leaves of *M. crenata* were collected in Surabaya, East Java, Indonesia. The collected plant materials were washed thoroughly with water, then dried carefully under shade, at room temperature so as to retain their fresh green color, and also to prevent decomposition of active compounds (Sarker *et al*. 2006).

Extraction and Fractination

N-hexane and ethyl acetate as solvent and mobile phase, maseration chamber, vacum column chromatography with diameter 6,5 cm and hight 6,5 cm, TLC plate silica gel 60 F254 Merck, silica gel 60 G Merck, H_2SO_4 10% reagent, and 254 nm also 366 nm UV lamp.

Method

Extraction and Fractination

The leaves of *M. crenata* were extracted with *n*-hexane through maseration method. The *n*-hexane extract of *M. crenata* leaves then separated using vacuum column chromatography on 70 g silica gel 60 G Merck as the stationary phase, and *n*-hexane : ethyl acetate as the mobile phase at gradient eluation.

Analysis with GC-MS

The *n*-hexane extract and fractions were analyzed using an HP gas chromatography model 6890 coupled to an MSD detector. Analysts were separated on an HP-5MS capillary column Agilent 19091S-433 (30.0m X 250µL X 0.25µL) with constant flow of carrier gas 1.0mL/min and initial pressure 11.6 psi, and then applying the following temperature program: 120°C for 2min, 120-200°C at 10°C/min, and 200°C for 10min. Mass detector conditions were acquisition mode scans, source temperature are minimum 230°C and maximum 250°C. Carrier gas was helium at 1.0 mL/min. The tentative identification of volatile components was achieved by comparing the mass spectra with the data system library (NIST02) and (Wiley275), supported by retention index data, which were compared with available literature retention indices.

RESULTS AND DISCUSSION

A total of 1.5 kg green powder was extracted with *n*-hexane to produce 22 g extract. After that, 3.5 g of *n*-hexane extract was separated using vacuum column chromatography on 70 g silica gel 60 G Merck as the stationary phase, and *n*-hexane : ethyl acetate as the mobile phase at gradient eluation. The separation process resulted in four fractions, there are fraction 1 (2,236 mg), fraction 2 (1,100 mg), fraction 3 (403 mg), and fraction 4 (96 mg). The separation process of *n*-hexane extract into several fractions using dry column vacuum chromatography needs to be done before analysis by GC-MS. This separation serves to improve the detection capabilities of GC-MS. Some peak that cannot be detected in the extract can be detected in fractions. This is because the compounds in each fraction, which are separated and identified by GC-MS, is not as much as the compounds in the extract.

The results of GC-MS analysis of the *n*-hexane extract and four fractions of *M. crenata* leaves show some compounds contained as listed in Table I. In Figure 2, we can see the Total Ion Chromatogram (TIC) of *n*-hexane extract and four fractions of *M. crenata* leaves. Mass spectra of each peak of extracts and fractions are further identified and matched with a data system library NIST02 and Wiley275. Each peak is identified with match factor should more than 85%. For examples, see figure 3. Figure 3 is mass spectra of peak from *n*-hexane extract that has a retention time (a) 11,996 and (b) 14,725, its appropriate with mass spectra of (a) neophytadiene with match factor 99%, and (b) *n*-hexadecanoic acid with match factor 98% .

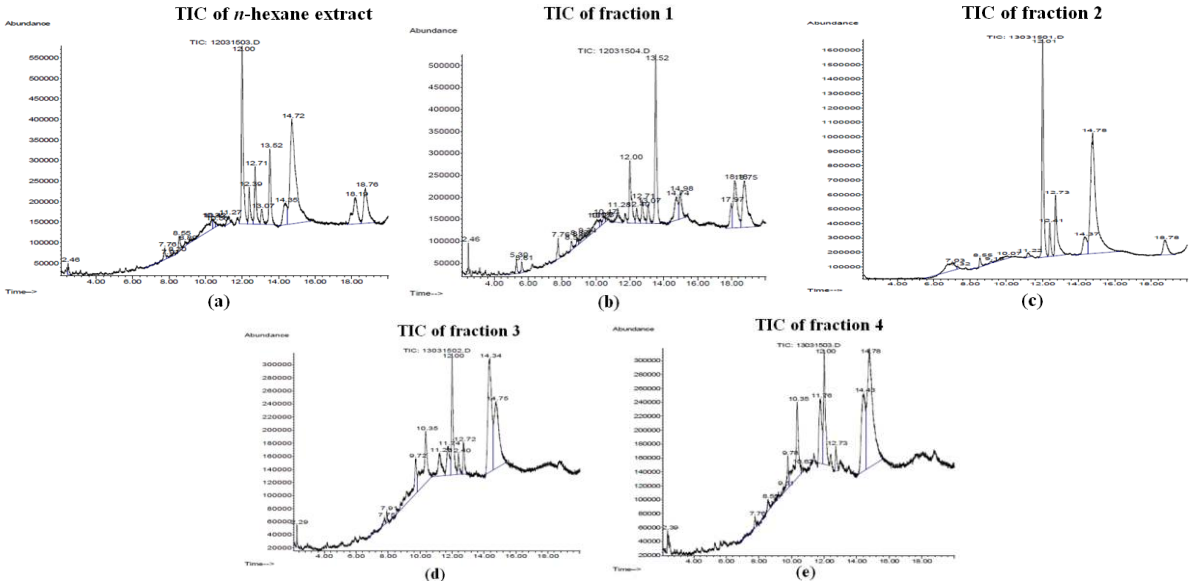

Figure 2. TIC of (a) *n*-hexane extract, (b) fraction 1, (c) fraction 2, (d) fraction 3, and (e) fraction 4 of *M. crenata* leaves.

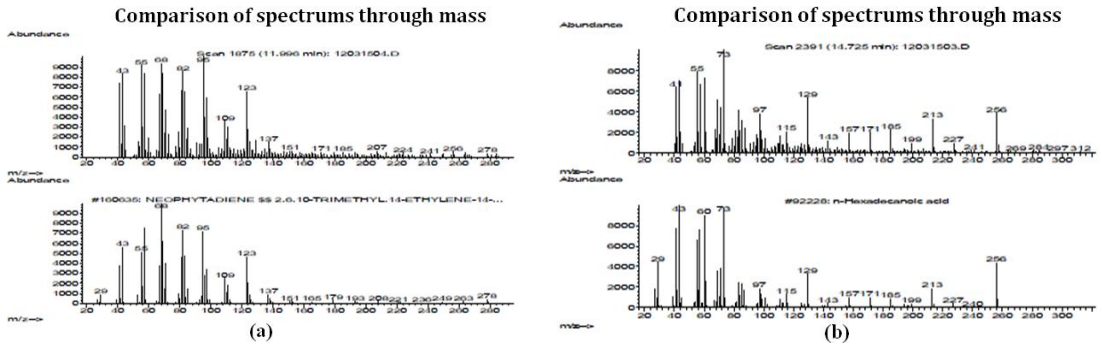

Figure 3. Comparison of spectrums through mass finder. (a) neophytadiene and (b) *n*-hexadecanoic acid

In general, the result of GC-MS show that the *n*-hexane extract of *M. crenata* leaves predicted contains many volatile group compounds such as monoterpenoid and diterpenoid, and fatty acid group compounds. The *n*-hexane extract and fractions of *M. crenata* leaves contain some diterpenoid compounds such as neophytadiene that have activity as antipyretic, analgesic, antiinflammatory, antimicrobial, and antioxidant; dihydroactinidiolide that have function as fragnance or pheromon in animal; phytol that have activity as antiinflammatory, antimicrobial, antioxidant, and diuretic; fatty acid compounds such as palmitic acid that have activity as antioxidant, hypocholesterolemic, antiandrogenic, and 5-Alpha reductase inhibitor; oleic acid that have activity as cancer preventive and antiandrogenic; elaidic acid that have activity as anticholesterol and lower LDL; and also other unknown compounds. Some of predicted compounds are impossible found in extract and fractions of natural product. For example, methyl ester form of fatty acid is normally detected after saponification process in laboratory work.

Through correlation analysis between TIC GC-MS and literature study, it can be concluded that palmitic acid is estrogen-like substance in *M. crenata* leaves that suspected have a role as antiosteoporotic agent, especially in improvement of osteogenesis (Kim *et al*. 2013). The mechanism of palmitic acid in improving osteogenesis occurs through enhancement of osteoblast's transcription factor in differentiation process such as runt-related transcription factor-2 (runx-2) and osterix (osx) significantly (Kim *et al*. 2013).

Table I. Predicted compounds of *M. crenata* leaves

Sample	Peak	Rt	% Area	Compounds	MF	MW	Function
n-Hexane Extract	1	2,46	0,54	Unknown			
	2	7,76	0,91	Unknown			
	3	7,91	0,18	Unknown			
	4	8,29	0,41	Unknown			
	5	8,55	1,60	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)- / dihydroactinidiolide	86	$C_{11}H_{16}O_2$	Flavour component, fragnance, pheromone in animal, indu gen expression
	6	8,89	0,53	Unknown			
	7	10,35	4,73	Unknown			
	8	10,42	0,77	Unknown			
	9	10,56	0,27	9-Octadecenoic acid, (E)- / Octadec-9-enoic acid / Elaidic acid	95	$C_{18}H_{34}O_2$	Increases plasma cholesterylester transfer protein activit which lowers HDL cholesterol
	10	11,28	1,10	Unknown			
	11	12,00	17,56	Neophytadiene / 7,11,15-trimethyl-3-methylidenehexadec-1-ene	99	$C_{20}H_{38}$	Antipyretic, analgesic, anti-inflammatory, antimicrobial, antioxidant
	12	12,39	3,38	Unknown			
	13	12,72	5,61	Unknown			
	14	13,06	1,65	Oleic Acid	96	$C_{18}H_{34}O_2$	Cancer preventive, anemiagenic, insectifuge, antiandrogen dermatitigenic
	15	13,52	7,64	Hexadecanoic acid, methyl ester	97	$C_{17}H_{34}O_2$	Antioxidant, flavor, hypocholesterolemic pesticide, 5-alpha reductase inhibitor
	16	14,35	4,48	Unknown			
	17	14,72	33,30	*n*-Hexadecanoic acid / Palmitic acid	98	$C_{16}H_{32}O_2$	Antioxidant, hypocholesterolemic, nematicide, pesticide, lubr antiandrogenic, flavor, hemolytic, 5-Alpha reductase inhibitor
	18	18,19	7,09	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	94	$C_{19}H_{32}O_2$	Antiinflammatory, hypocholesterolemic, cancer preventiv hepatoprotective, nematicide, insectifuge, antihistaminic antiarthritic, anticoronary, antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic
	19	18,75	8,24	Unknown			

Sample	Peak	Rt	% Area	Compounds	MF	MW	Function
Fraction 1	1	2,46	1,39	2,4-Heptadienal, (E,E)-	94	$C_7H_{10}O$	Lower LDL, antioxidant
	2	5,31	1,66	Unknown			
	3	5,61	1,13	Unknown			
	4	7,76	2,08	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	93	$C_{13}H_{20}O$	Fragnance, antioxidant, inhibition proliferation of cancer cell
	5	8,55	0,94	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)- / dihydroactinidiolide	89	$C_{11}H_{16}O_2$	Flavour component, fragnance, pheromone in animal, inducing gen expression
	6	8,87	0,86	Unknown			
	7	8,99	0,44	Unknown			
	8	9,34	1,13	Unknown			
	9	10,11	3,91	Unknown			
	10	10,27	0,71	9-Octadecenoic acid, (E)- / Octadec-9-enoic acid / Elaidic acid	95	$C_{18}H_{34}O_2$	Increases plasma cholesterylester transfer protein activity which lowers HDL cholesterol
	11	10,47	1,25	Cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpentyl)-	90		
	12	10,61	0,22	Unknown			
	13	11,28	2,51	6-Octadecenoic acid, (Z)-	94	$C_{18}H_{34}O_2$	Cancer preventive, insectifuge
	14	12,00	9,60	Neophytadiene / 7,11,15-trimethyl-3-methylidenehexadec-1-ene	99	$C_{20}H_{38}$	Antipyretic, analgesic, anti-inflammatory, antimicrobial, antioxidant
	15	12,40	2,07	Unknown			
	16	12,72	3,36	Unknown			
	17	13,06	2,63	Oleic Acid	95	$C_{18}H_{34}O_2$	Cancer preventive, anemiagenic, insectifuge, antiandrogenic, dermatitigenic
	18	13,52	20,49	Hexadecanoic acid, methyl ester	97	$C_{17}H_{34}O_2$	Antioxidant, flavor, hypocholesterolemic pesticide, 5-alpha reductase inhibitor
	19	14,74	6,08	Unknown			
	20	14,98	4,67	Cyclopentadecane	86		
	21	17,97	3,86	10,13-Octadecadienoic acid, methyl ester	99		
	22	18,18	12,92	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	88	$C_{19}H_{32}O_2$	Antiinflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antiarthritic, anticoronary, antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic
	23	18,75	16,10	Unknown			

Sample	Peak	Rt	% Area	Compounds	MF	MW	Function
Fraction 2	1	7,03	4,43	Tetradecanoic acid / Myristic acid	86	$C_{14}H_{28}O_2$	Antioxidant, cancer preventive, cosmetic, hypercholesterolemic, nematicide, lubricant
	2	7,32	0,25	Unknown			
	3	8,55	0,83	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	93	$C_{11}H_{16}O_2$	Flavour component, fragnance, pheromone in animal, inducing gen expression
	4	9,18	0,39	Unknown			
	5	10,07	1,47	Unknown			
	6	11,22	0,68	Unknown			
	7	12,01	24,05	Neophytadiene / 7,11,15-trimethyl-3-methylidenehexadec-1-ene	99	$C_{20}H_{38}$	Antipyretic, analgesic, and anti-inflammatory, antimicrobial, antioxidant
	8	12,41	3,52	Unknown			
	9	12,73	9,85	Phytol	90	$C_{20}H_{40}O$	Antimicrobial, anticancer, cancer preventive, diuretic antiinflammatory
	10	14,37	4,92	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	89		
	11	14,78	45,18	*n*-Hexadecanoic acid / Palmitic acid	99	$C_{16}H_{32}O_2$	Antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, flavor, hemolytic, 5-Alpha reductase inhibitor
	12	18,78	4,43	Unknown			
Fraction 3	1	2,29	0,92	Unknown			
	2	7,75	0,66	Unknown			
	3	7,91	0,61	Unknown			
	4	9,72	9,57	Unknown			
	5	10,35	13,00	Unknown			
	6	11,23	5,27	Unknown			
	7	11,74	5,28	Unknown			
	8	12,00	12,69	Neophytadiene / 7,11,15-trimethyl-3-methylidenehexadec-1-ene	99	$C_{20}H_{38}$	Antipyretic, analgesic, and anti-inflammatory, antimicrobial, antioxidant
	9	12,40	1,89	9,12-Octadecadienoic acid (Z,Z)- / Linoleic acid	87	$C_{18}H_{32}O_2$	Antiinflammatory, nematicide, insectifuge, hypocholesterolemic, cancer preventive, hepatoprotective, antihistaminic, antiacne, antiarthritic, antieczemic, 5-alpha reductase inhibitor, antiandrogenic, anticoronary
	10	12,72	2,96	Unknown			
	11	14,34	26,03	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	89		
	12	14,75	21,12	*n*-Hexadecanoic acid / Palmitic acid	95	$C_{16}H_{32}O_2$	Antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, flavor, hemolytic, 5-Alpha reductase inhibitor

Sample	Peak	Rt	% Area	Compounds	MF	MW	Function
Fraction 4	1	2,39	0,14	Unknown			
	2	7,76	0,85	Unknown			
	3	8,55	0,82	Unknown			
	4	9,51	1,09	9-Octadecenoic acid, (E)- / Octadec-9-enoic acid / Elaidic acid	95	$C_{18}H_{34}O_2$	Increases plasma cholesterylester transfer protein activity which lowers HDL cholesterol
	5	9,78	2,88	Unknown			
	6	10,35	13,13	Unknown			
	7	10,62	0,33	Tetrahydroxycyclopentadienone	86		
	8	11,76	10,01	(-)-Loliolide	87	$C_{11}H_{16}O_3$	Germination inhibitory, anticancer, immunosuppressive activity
	9	12,01	12,46	11,13-dimethyl-12-tetradecen-1-ol acetate	86		
	10	12,73	1,99	Unknown			
	11	14,43	17,21	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	92		
	12	14,78	39,07	*n*-Hexadecanoic acid / Palmitic acid	97	$C_{16}H_{32}O_2$	Antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, flavor, hemolytic, 5-Alpha reductase inhibitor

CONCLUSIONS

Through GC-MS analysis, it is known that *M. crenata* leaves contain many volatile group compounds such as monoterpenoid, diterpenoid, and fatty acid group compounds that have various activity. Palmitic acid, one of fatty acid contained in *M. crenata* leaves, is suspected to have a role as antiosteoporotic agent, especially in improvement of osteogenesis. Therefore, further research is need to be done to prove it's activity.

REFERENCES

Afriastini, J.J. 2003. *Marsilea crenata* Presl. in Cryptograms : ferns and fern allies.

Nurjanah, Azka, A., Abdullah, A., 2012. Aktivitas antioksidan dan komponen bioaktif semanggi air (*Marsilea crenata* Presl.). Jurnal Inovasi dan Kewirausahaan. 1: 152-158.

Steenis, C.G.G.J.V. 1975. Flora Untuk Sekolah di Indonesia. Halaman 80-81.

Bhardwaja, T.N. 2008. Marsileaceae : An amphibious heterosporous group of ferns, the persisting enigma. Asian Journal of Experimental Science. 22: 189-191.

Yacoeb, A.M., Nurjanah, Arifin, M, Sulistiono, W, Kristiono, S.S. 2010. Description and histological changes in the chemical composition of the leaves and stalks of *Marsilea crenata* Presl. by boiling. Journal of Fishery Products Processing Indonesia XII. 2: 81-95.

Laswati, H. 2011. Green clover potentiates delaying the increment of imbalance bone remodelling process in postmenopausal women. Folia Medica Indonesiana. 47: 112-117.

Yang, T.S., Wang, S.Y., Yang, Y.C., Su, C.H., Lee, F.K., Chen, S.C., *et al*. 2012. Effects of standardized phytoestrogen on Taiwanese menopausal women. Taiwanese Journal of Obstetrics & Gynecology. 51: 229-235.

Ososki, A.L., Kennelly, E.J. 2003 Phytoestrogens: a review of the present state of research. Phytotherapy Research. 17: 845-869.

Elezabeth, V. and Arumugam. 2014. GC-MS analysis of bioactive constituents of *Indigofera suffruticosa* leaves. Journal of Chemical and Pharmaceutical Research. 6: 294-300.

Kumar, S., Samydurai, Ramakrishnan, Nagarajan, N. 2014. Gas chromatography and mass spectrometry analysis of bioactive constituens of *Adiantum Capillus-Veneris* L.. International Journal of Pharmacy and Pharmaceutical Sciences. 6: 60-63.

Bai, S., Seasotiya, L., Malik, A., Bharti, P., Dalal, S. 2014. GC-MS analysis of chloroform extract of *Acacia nilotica* L. leaves. Journal of Pharmacy and Phytochemistry. 2: 79-82.

Sarker, S.D., Latif, Z., Gray, A.I. 2006. An overview of natural product isolation. Natural Product Isolation 2nd Ed. Totowa : Humana Press Inc.: 1-26.

Shettima, A.Y., Karumi, Y., Sodipo, O.A., Usman, H., Tijjani, M.A. 2013. Gas chromatography – mass spectrometry (GC-MS) analysis of bioactive components of ethyl acetate root extract of *Guiera senegalensis* J.F. Gmel. Journal of Applied Pharmaceutical Science. 3: 146-150.

Sermakkani, M. and Thangapandian. 2012. GC-MS analysis of *Cassia Italica* leaf methanol extract. Asian Journal of Pharmaceutical and Clinical Research 5: 90-94.

Venkata, R.B., Samuel, L.A., Pardha, S.M., Narashima, R.B., Naga, V.K.A., Sudhakar, M., *et al*. 2012. Antibacterial, antioxidant activity and GC-MS analysis of *Eupatorium odoratum*. Asian Journal of Pharmaceutical and Clinical Research. 5: 99-106.

Devi, J.A.I. and Muthu, A.K. 2014. Gas chromatography - mass spectrometry analysis of bioactive constituents in the ethanolic extract of *Saccharum Spontaneum* Linn.. International Journal of Pharmacy and Pharmaceutical Sciences. 6: 755-759.

Dar, S.A., Ganai, F.A., Yousuf, A.R., Balkhi, M., Bhat, T.M., Bhat, F.A. 2012. Bioactive potential of leaf extracts from *Urtica dioica* L. against fish and human pathogenic bacteria. African Journal of Microbiology Research. 6: 6893-6899.

Dabdoub, M.J., Silveira, C.C., Lenardao, E.J., Viana, L.H., Kawasoko, C.Y., Baroni, A.C.M., *et al*. 2009. Total synthesis of (±)-dihydroactinidiolide using selenium-stabilized carbenium ion. Tetrahedron Letters. 50: 5569-5571.

Joshi-Barve, S., Barve, S.S., Amancherla, K., Gobejishvili, L., Hill, D., Cave, M., *et al*. 2007. Palmitic acid induces production of proinflammatory cytokine interleukin-8 from hepatocytes. Hepatology Journal. 46: 823-830.

Hadi, M.A.M., Zhang, F., Wu, F., Zhou, C., Tao, J. 2013. Advances in fruit aroma volatile research. Molecules Journal. 18: 8200-8229.

Shan, Wu, L., Cheng, P., Yu, J., Zhang, A., Zha, J., Liu, J., Wang, L., Di, W., Hu, M., Qi, H., Li, Y., Ding, G. 2010. Correlation of obesity and osteoporosis: Effect of free fatty acids on bone marrow-derived mesenchymal stem

cell differentiation. Experimental and therapeutic medicine. 1: 603-610.

Kim, J., Park, Y., Lee, S., Park, Y. 2013. Trans-10,cis-12 Conjugated linoleic acid promotes bone formation by inhibiting adipogenesis by peroxisome proliferator activated receptor-γ-dependent mechanisms and by directly enhancing osteoblastogenesis from bone marrow mesenchymal stem cells. Elsevier : Journal of Nutritional Biochemistry. 24: 627-679.