Sindh Univ. Res. Jour. (Sci. Ser.) Vol. 47 (3) 581-584 (2015)

SINDH UNIVERSITY RESEARCH JOURNAL (SCIENCE SERIES)

Chemical Analysis of Fatty Acids Elementlogy and Total Protein of (*Mentha Arvensis L.*)

S. K. KHANZADA[++], A. K. KHANZADA, F. HUSSAIN*, A. R. KHASKHELI**

Institute of Plant Sciences, University of Sindh, Jamshoro, Sindh, Pakistan.

Received 5th November 2014 and Revised 6th January 2015

Pakistan has been well-known for its rich variety of medicinal plants. For this study, Mentha arvensis L. medicinal plants belong to family Lamiaceae from methanolic extract of *Mentha arvensis L*. collected from (Shaheed Benazir Abad) (NawabShah), Sindh Pakistan. The highest saturated fatty acids were present in *Mentha arvensis L.* Behenate, Palmilate 4.90% unsaturated fatty acids of *Mentha arvensis L*. in Heptadecadienoate 19.59 % were observed. Total 29 compounds were isolated the extracts of the *M. arvensis L.* plant saturated and unsaturated fatty acids have been isolated and identified using GC-MS method, and 6 elements viz., Copper, 11.17(mg/kg)Iron, 5.8(mg/kg)Sodium,60.20 mg/kg) Magnesium, 16.20 (mg/kg) Potassium, 10.31(mg/kg)and Zinc 12.47(mg/kg)were isolated from, *Mentha arvensis L* were isolated from, *Mentha arvensis L* accumulation of Potassium was the lowest in *Mentha arvensis L* accumulation. Total protein in *M. arvensis L* was 42.44 %, as established by kjeldhal method. Protocol of ISI-24-1-e, was under of determination of total nitrogen, which was calculated using a nitrogen conversion factor of 6.25. The aim of present study was investigated the presence of fatty acids, elements and protein, give information about this plant contain medicinally important compounds are present and informative knowledge for the traditional medicines for the treatment of different diseases.

Keywords: *Mentha arvensis L.*, fatty acids, elements total, protein, medicinal plant

1. INTRODUCTION

Mint *(Mentha arvensis L)* belong to the family Lamiaceae. (Labiatae) *Mentha arvensis L* medicinal. It is locally known as pudina in Urdu, with English name as Mint cultivate abundantly found in throughout Pakistan. The aerial parts of the herb containing a great amount of fragrance chemicals resembling menthol, linalool, menthone, isomenthone, menthofuran, and piperitenone oxide which are used in pharmaceutical, food, flavour, cosmetics, beverages and industries. (Khanuja, 2007; Patra *et al.,* 2002 Verma, *et al* 2010. Lamiaceae herb family consists of more than 252 genus and 7000 species (Hedge, 1992). This family is known for the wealth of species with medicinal properties, which have been used since early period and numerous of these species are universal in Mediterranean region (Ali *et al.,* 2000). WHO information that 70% of the people of developed countries depends on natural product drugs for health care (Kumar *et al* 2004). *M.* arvensis *L* is distributed throughout the western Himalayas and is cultivated throughout world for use as a vegetable. *Mentha* arvensis *L*. is used as a carminative, anti-spasmodic, anti peptic ulcer agent, and has been given to treat indigestion, skin diseases, coughs and colds in folk medicine. According to many researchers the plant have 90% mint oil, monoterpenes ,(menthone, menthofuran, methyl acetate cineole sesquiterpenes, flavonoids, luteolin, menthoside, phenolic acids triterpenes; tocopherols; betaine; rosmarinic acid; cyclenes; tannin; (Pino 1996, Liest, 1998). *Mentha* arvensis *L*. is used as a carminative and an expectorant. *M. arvensis* L. is mostly useful in care for headaches, cough sore throat, colic and vomiting. *M. arvensis* L serves as a good blood cleanser, antiseptic, anti-bacterial and leaves are used in care for insect bites.

All chemical compounds used in the medicinal formulas are obtained from plants from long ago, due to the presence of alkaloids, flavonoids, glycocides etc. Anti-inflammatory agent like flavonoids, saponins, triterpenes, carotenoids, fatty acid, eloeostearic acid, are also one of the reason of using plant extracts as medicine. (Mors *et al.* 2000, Ferreira *et al.* 1992. Lindahl 1997. Singh 1997; Chisholm 1967). Common source of medication are plants in traditional preparation or as pure active constituents. All types of treatment methods e.g. Hikmat, Ayurvedic, Allopathic and Homeopathic, are based on plants commercially (Mehmood *et al.* 2003). Life is totally dependent on plants (Iqbal 2006; Wani *et al.,* 2010). A number of physiological important substances are synthesized by the plants (Kretovich, 2005). Plant role in human nutrition is paramount especially in malnourished communities of developing countries (Conway 1999, Mohamed, 1992; Yanez *et al.,* 1995). Fatty acids are those organic compounds which are very important regarding human diet as certain diseases are prevented by their omega-3 Fatty acids and poly unsaturated Fatty acids (Burgess *et al.,* 1991; Wise *et al* 1994).

2. MATERIALS AND METHODS.

Collection of plant material: *(Mentha arvensis L)* were collected from (Shaheed Benazir Abad) (NawabShah), March –April 2010. Reference samples were identified by through literature flora of Pakistan. (Nasir and Ali

[++]Corresponding author: E-mail drsaminakabir@gmail.com

*Department of Agriculture and Agribusiness Management, University of Karachi-75270

*Department of Pharmacy, Shaheed Mohtarma Benazir Bhutto Medical University, Larkana, Sindh, Pakistan

1971). The collected plant materials were washed with tap water followed by distilled water and dried in shade at room temperature for 20 days.

Extraction: The dried plant was chopped into small pieces and was dipped into 2 litre ethanol (EtOH) for about one month at room temperature. The ethanolic extract was filtered and evaporated under reduced pressure at below 40°C using rotary evaporator, which yielded dark green gummy residue. The extracts were then partitioned with Ethyl acetate (EtOAc) and water which this procedure was repeated 3 times.

Column chromatography (CC): The residue containing fatty acids fraction was separated on chromatograph over silica gel. (70-230 mesh Merck) column. The column was first eluted with n-hexane and thereafter chloroform was added in order of increasing polarity. First fraction was eluted with pure hexane, fraction "A" was eluted from hexane: chloroform (85:15), fraction "B" from hexane: chloroform (80:20), fraction "C" from hexane: chloroform (75:25), and fraction "D" from hexane: chlorofrom (70:30).

Esterification: All fractions (A-D) were esterified with diazomethane, 0.5 mg of each fraction was dissolved in MeOH and 0.5 ml of diazomethane was added. The reaction mixture was kept overnight at room temperature (28°C) and was then evaporated.The methylated fatty acid fractions were analyzed by GC-MS.

Gas chromatography-mass spectrometry (GC-MS): The fatty acids (mathly ester) fraction werefinally analysed and identified by G.C-MS, the anysis was performe on was performed on JEOL JMS 600H Agilest 689 ON, equipped with 30 m×0.32 ZP-5 MS column, stationary phase coating 0.25µm. The column temperature was kept at 70°C for 2 min with increased at the rate of 4°C per min up to 250°C. Injector temperature 250°C, split ratio 1:45, the carrier gas (N_2/QHe flow rate 1.0ml/min. (Khanzada *et al.*, 2008.a.b, 2013, 2014).

Elemental assay:

The samples were investigated for elemental analysis by using atomic absorption spectrophotometer (AAS), Hitachi Ltd. 180-50.S.N5721. Appropriate working standard solution was prepared for each element (Khanzada *et al.*, 2008.a.b, 2013 , 2014).

Total protein analysis by kjeldhal method: The sample was digested in H_2SO_4 (30 ml) in the presence of catalyst $CuSO_4$ (1g) and K_2SO_4 (10g), after digestion Sodium hydroxide (NaOH, 33%) was added followed by steam distillation, the distillate was collected in 20 ml boric acid (4%). Then nitrogen content was determined by using titration with HCl (0.01N). A factor of 6.25 was used to evaluate total protein contents (Khanzada *et al.*, 2008.a.b, 2013, 2014,2015).

Total protein analysis: Protocol of ISI-24-1-e, was found determination of total nitrogen, which was calculated using a nitrogen conversion factor of 6.25 (Khanzada *et al.*, 2008.a.b, 2013, 2014, 2015).

3. RESULT AND DISCUSSION

The present investigation demonstrated the usefulness of *Mentha arvensis L.* plant extract. The *Mentha arvensis L.* were analyzed for their fatty acid composition The results obtained have been presented in. (Table 1,2) 29 total compound were isolated, 6 SFAs and 23, UFAs and these tables also showed the relative retention time (RRT) and relative percentage of occurrence of their. The lowest and highest relative % of saturated fatty acids present Mentha arvensis L. in the range of Methyl-n-Heptenoate (2.18%), n-Hexadecanoate, (4.90%), and lowest and highest unsaturated fatty acids Methyl (all cis)-eicosapentaenoate (0.91%) the ranges of Heptadecadienoate (19.59%), total SFAs percentages 21.77% and total UFAs fatty acid (78.22).

In pervious work of *Pithecellobium dulce Benth.* fatty acid analysis 9 saturated and 17 unsaturated fatty acids were identified and on *Calotropis procera* medicinal plant 18 different fatty acids (FAs) saturated fatty acids were in greater quantity (52.92%) than unsaturated fatty acids (47.08%).(Khanzada *et al* 2008 a,b, 2013). The comparison with other fatty acid highest in stem *Calotropis procera* (65.9%) with 58.7% whole plant extract were, that *Calendula officinalis L.* flowers, extract which contain total 37 fatty acids among which 13 were saturated 24 unsaturated The unsaturated fatty acid were present in higher than saturated (Khanzada *et al* 2014).

Plant yield have component of phytomedicines since ancient time, and obtained from bark ,leaves flowers roots, fruits ,seeds (Criagg, *et al,* 2001). previously reported research work on medicinal plants fatty acids from Calotropis procera of 18 different fatty acids (FAs) saturated fatty acids were greater quantity (52.92%) than unsaturated fatty acids (47.08%). The comparison with other fatty acids found to be higest in the stem of Calotropis procera (65.9%) with 58.7% whole plant extract, (Kalita *et al.,* 2004). (Khanzada *et al*. 2013). (Khanzada *et al.* 2008. a, 2008.b) , that Calendula officinalis L. flowers, extract which contain total 37 fatty acids among which 13 were saturated 24 unsaturated The unsaturated fatty acids present in higher (55.14%) than saturated fatty acids acid, SFAs (42.85%). The largest amount of (SFAs) 16.68%. (Kalita *et al.,* 2004). (Khanzada *et al.* 2014).

Total protein analysis: The total protein contents isolated of whole plant Mentha arvensis L was 42.44 %, previously reported work in Tamarindus indica: Jamshoro 15.6%, Nawabshah Distt, 10.8%. Hyderabad

Distt, 8.7%. (Khanzada *et al.*, 2008.a,b) The total protein content was found to be lower when compared to an earlier report T. indica 14% (Arinathan *et al.,* 2003). Seed protein 6.9% (M Pugalenthi *et al.,* 2004.) If we compared previously reported work in different medicinal plants protein from Calotropis procera protein (7.87-14.63% the highest total protein was reported in Calotropis procera in leaf extracts 23.94, stem 8.94, bark 12.69 (Kalita *et al.,* 2004). Watermelon seed protein the high-quality dietary and determined property of proteins, such as used in food formulations property recently reported by (Ali *et al.* 2011). Total protein contents in seeds of Pithecellobium dulce Benth .the ranged between 67.11, while stems, roots and leaves contains, 15.72%, 10.58% and 13.75% correspondingly and flowers and fruits 14.76% and 10.50%, recent work of Pithecellobium dulce Benth seeds have rich sources of protein. (Khanzada *et al* 2013). In Calotropis procera of Sindh,the higest percentage was 50.80% of dry weight (Daulatpur), 32.11% (NawabShah), 25% (Hyderabad) and (Jamshoro), and 29.45% from different sites (Khanzada *et al.,* 2008.b). Total protein in Calendula officinalis L. flowers is reported from seed 30.20 %. (Khanzada *et al.,* 2014).

Elements analysis: Six elements viz., Copper, 11.17(mg/kg) Iron, 5.8(mg/kg) Sodium,60.20 mg/kg) Magnesium, 16.20 (mg/kg)Potassium, 10.31(mg/kg)and Zinc 12.47(mg/kg)were isolated from, Mentha arvensis L were isolated from, *Mentha arvensis L* Accumulation of Potassium 10.31(mg/kg) was the lowest in *Mentha arvensis L* while Sodium present with highest 60.20 (mg/kg)accumulation .If we compare with others, Magnesium 60.1 is present in *T.indica* and minimum ratio in Copper. 0.76. (Khanzada *et al.,* 2008.a, Ishola et al.,1990); Sodium 28.83 Calcium 315.28 Potassium 248.56, Magnesium 285.14 Phosphorus 369.47, Iron 7.14, Copper 0.59, Zinc 6.94 Manganese 0.81 (Pugalenthi *et al.,* 2004). Magnesium 71.0, Iron 2.0 Copper 2.0, Sodium 8.0 Potassium (mg) 270.0 (Lewis *et al* 1964; Anon., 1976; Duke 1981) detected mineral composition. Calcium, 36.6, Copper 2.10, Manganese 12.1, Sodium 8.90, Iron 45.5, Zinc 7.00, Potassium 1308, Magnesium, 104 (Ajayi *et al.,* 2006). *T. indica* seeds have Calcium, 10.00. Potassium 21.0. Sodium, 2.1. Magnesium, 15.0. Iron, 75.9. (Yusuf *et al,* 2007) and Copper 9.09, Magnesium, 1.153, Sodium, 62.1, Iron 31.7, Zinc 13.2, Potassium 6.54 (Robert *et al*., 2005).

Table 1. Saturated fatty acids of *Mentha arvensis L.* analyzed as methyl ester

S.No	Systematic Name	Common Name	MolecularFormula	Mol.Wt	R.R.T	Rel.%Ag
1	Methyl-n-Heptenoate	Heptylete	$C_8H_{16}O_2$	144	17.37	2.18
2	Nonanoate	Laurate	$C_{13}H_{26}O_2$	214	19.63	3.40
3	n-Hexadecanoate	Palmilate	$C_{17}H_{34}O_2$	270	28.7	4.90
4	n-Octadecanoate	Stearate	$C_{19}H_{38}O_2$	298	32.47	2.99
5	n-Docosanoate	Behenate	$C_{23}H_{46}O_2$	354	38.68	4.90
6	n-Hxocosanoate	Cerotate	$C_{27}H_{54}O_2$	410	43.68	3.40
Total						21.77

Table 2. Unsaturated fatty acids of *Mentha arvensis* L. analyzed as methyl ester.

S.NO	SYSTEMATIC NAME	COMMON NAME	MOLECULAR FORMULA	MOL. Wt.	R.R.T	Rel. %ag
1.	Dodecenoate	Lauroleic	$C_{12}H_{22}O_2$	198	25.97	1.48
2	Tridecadienoate	Tridecadienooate	$C_{13}H_{22}O_2$	210	58.88	1.62
3	Tridecatrienoate	Tridecatrienoate	$C_{14}H_{22}O_2$	222	18.67	2.36
4.	Methyl-2-Tridecynote	Tridecynote	$C_{14}H_{24}O_2$	224	28.12	1.33
5	Methyl tricosenoate	Decylacrylate	$C_{14}H_{26}O_2$	226	29.32	1.63
6	2,4,5Tetradecatrienoat	Tetradecatrienoat	$C_{15}H_{24}O_2$	236	25.28	2.72
7.	Hexadecenoate	Palmileate	$C_{17}H_{32}O_2$	268	50.43	1.9
8	Heptadecadienoate	Heptadecadienoate	$C_{18}H_{34}O_2$	280	39.53	19.59
9.	Octadecadienoate	Octadecadienoate	$C_{19}H_{34}O_2$	294	36.42	2.99
10.	10-Octadecenoate	Oleate	$C_{19}H_{36}O_2$	296	31.97	3.81
11	Nenodecenoate	Nenodecenoate	$C_{20}H_{38}O_2$	310	33.73	0.95
12	Methyl(all cis)-eico-sapentaenoate	Eicosapentaenoate	$C_{21}H_{36}O_2$	316	61.7	0.91
13	Eicosadienoate	Eicosadienoate	$C_{21}H_{38}O_2$	322	47.45	1.63
14	9-Eicosenoate	Gadoleate	$C_{21}H_{40}O_2$	324	45.83	3.67
15	Heneicosenote	Heneicosenote	$C_{22}H_{42}O_2$	338	37.12	5.55
16	Methyl-tricosenote	Methyl-tricosenote	$C_{24}H_{46}O_2$	366	40.22	5.55
17	Tetracosadienoate	Tetracosadienoate	$C_{25}H_{46}O_2$	378	41.68	4.76
18	n-Pentacosenoate	Pentacosenoate	$C_{25}H_{48}O_2$	380	45.13	2.99
19	n-Hexacoseoate	Hexacoseoate	$C_{26}H_{50}O_2$	394	42.75	3.4
20	Methyl hexacosenate	Hexacosenate	$C_{27}H_{52}O_2$	408	51.08	1.63
21	Nonacosenoate	Nonacosenoate	$C_{30}H_{56}O_2$	450	43.25	3.67
22	Methyl-n-dotriacontanoate	Dotriacontanoate	$C_{33}H_{64}O_2$	492	54.55	1.77
23	n-Tritriacontanoate	Tritriacontanoate	$C_{34}H_{66}O_2$	508	57.28	2.31
TOTAL					78.22	

6 Saturated, 23 Unsaturated, ,Total compounds=29 ,Total %age of Saturated + Unsaturated fatty acid = 99.99 , Mol. wt=molecular weight, R.R.T=relative retention time, Re l% age=relative percentage

REFERENCE:

Ali, M. S., M. Saleem, Z. Ali and V. U. Ahmad. (2000). Chemistry of Zataria multifora (Lamiaceae). Phytochemistry. 55: 933-936.

Bharadwaj, S. D. (1984). Harvesting management of *Mentha citrata* Ehrh. in mid hill conditions of Himanchal Pradesh. *Indian Perfumer* 28: 38-41.

Court, W., R.C. Roy, (1993). Effect of harvest date on the yield and quality of the essential oil of peppermint. *Canadian Journal of Plant Science* 73: 815-824.

Calotropis Procera medicinal plant from Sindh, Pakistan. *Pak. J. Bot*., 40(5): 1913-1921.

Croteau, R. and W. D. Loomis, (1972). Biosynthesis of mono and sesquiterpenes in peppermint from mevalonate-2 ^{14}C. *Phytochemistry* 11: 1055-1066.

Farooqi, A.H.A., A. Mishra, and A. A. Naqvi, (1983). Effect of plant age on quality of oil in Japanese mint. *Indian Perfumer* 27: 80-82.

Gasic, O., N., Mitica-Dukic, and K. Borojevic, (1989). Variability of content and composition of essential oil in different genotypes of peppermint. *Biochemical Systematics and Ecology* 15: 335-340.

Hefendehl, F.W. and M. J. Murray, (1973). Monoterpene composition of chemotype of *Mentha piperita* having high limonene. *Planta Medica* 23: 101-106.

Khanuja, S.P.S. (2007). Aroma vision 2020: Technology path of cultivating today for a fragrant tomorrow. National convention & seminar on business enabling of aromatic plants & products at Dehradun, India. November 21-22.

Kofidis, G., A. Bosabalidis, and S. Kokkini, (2004). Seasonal variation of essential oils in a linalool rich chemotype of *Mentha spicata* grown wild in Greece. *Journal of Essential Oil Research* 16: 469-472.

Kumar, S., J. R. Bahl, and S. Sharma, (2000). Profiles of the essential oils of Indian menthol mint *Mentha arvensis* cultivars at different stages of crop growth in northern plains. *Journal of Medicinal and Aromatic Plants Sciences* 22: 774-786.

Khanzada S. K., A. Kabir and M. Baloch (2014). Isolation of Fatty Acid, Elements Protein Contents in Flowers of *Calendula Officinalis L.,* From Jamshoro, Sindh- *Sindh Univ. Res. Jour. (Sci. Ser.) Vol. 46 (4) 553-556.*

Khanzada, S. K., W. Shaikh, S. Sofia, T. G. Kazi, A. K. Khanzada and S.T.H. Sherazi. (2008a).Chemical constituents of *Tamarindus indica* L. medicinal plant in Sindh. *Pak. J. Bot*., 40(6): 2553-2559.

Khanzada, S. K., A. K. Khanzada, W. Shaikh, and S. A. Ali (2013). Phytochemical studies on *Pithecellobium dulce Benth.*a medicinal plant of Sindh, Pakistan, *Pak. J. Bot.*, 45(2): 557-561.

Khanzada S. K., A. K. Khanzada M. Baloch, A. R. Khaskheli and W. Shakih. *(2015)* Determination of Total Protein Contents from Medicinal Plants (Zygophyllaceae) Family Found in Pakistan. *Sindh Univ. Res. Jour. (Sci. Ser.) Vol.47 (1):41-44.*

Mojab, F., M. Kamalinejad, N. Ghaderi, H. R. Vanidipour, (2003). Phytochemicals screening of some species of Iranian plants. Iran. J. Pharm. Res., 3: 77-82.

Marotti, M., Dellaceae, V., Piccaglia, R. and Gioanelli, E. 1993. Effects of harvesting stage on the yield and essential oil composition of peppermint (*Mentha piperita* L.). *Acta Horticulturae* 344: 370-379.

Nasir E. and S. I. Ali (1972-1990). Flora of West Pakistan, Stewart Herbarium Gordon College, Rawalpindi and Department of Botany, University of Karachi.

Parekh, J., S. Chanda, (2007). Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. Afr. J. Biomed. Res., 10: 175-181.

Parekh, J., S. Chanda, (2008). Phytochemicals screening of some plants from western region of India. Plant Arch., 8: 657- 662.

Patra, N. K., B. Kumar, K. Shukla, P. Ram, (2002). Problems and issues of Agrotechnology transfer in menthol mint: A case study with variety Kosi. *Proceedings of First National Interactive Meet on Medicinal & Aromatic Plants CIMAP, Lucknow* pp: 440-443.

Ramesh L S. Londonkar and Pramod V. Poddar .(2009) .Studies on activity of various extracts of *Mentha arvensis* Linn against drug induced gastric ulcer in mammals. World J Gastrointest Oncol. 15; 1(1): 82–88.

Srivastava, R. K., A. K. Singh, A. Kalra, R.P. Bansal, V.K.S. Tomar, J.R. Bahl, and S. Kumar, (2000). Optimum crop age of menthol mint *Mentha arvensis* cv, *Kosi* crops in the Indo-gangetic plains for high yields of menthol rich essential oil. *Journal of Medicinal and Aromatic Plants Sciences* 22: 771-773.

Siddique, S. M. (1998). Nutritional composition of May grass. Pak. J. Sci. Ind. Res., 35(11): 66-70.

Verma, R. S., L. Rahman, R.K. Verma, A. Chauhan, and A. Singh. (2010). Essential Oil Composition of Menthol Mint (*Mentha arvensis)* and Peppermint (*Mentha piperita*) Cultivars at Different Stages of Plant

Growth from Kumaon Region of Western Himalaya. Journal of Medicinal and Aromatic Plants, 1(1): 13-18