Published online in http://ijam.co.in
ISSN: 0976-5921
International Journal of Ayurvedic Medicine, 2013, 4(4), 352-360

Investigation and chromatographic separation of some phenolic compounds from flowers of *Mentha longifolia* l. and *Mentha spicata* l. growing in Iraq

Research article

Fathi A. Al-Mandeel[1*]

Lecturer
Environmental and Pollution Control Research Center
University of Mosul, Iraq.

Abstract

Five of active phenolic compounds: Hydroquinone, Resorcinol, Coumarin, Vanillin and Gallic acid were separated and investigated by high performance liquid chromatography (HPLC) technique from the crude of ethanolic extract of *Mentha longifolia* L. and *Mentha spicata* L. and the retention time adopted as a base to investigation of those compounds.

The results showed that the crude extracts of the species under study contains of Hydroquinone also Gallic acid compounds while those extracts differed in their content of other studied phenolic compounds, however the crude extract of *M. longifolia* distinguished by presence of Resorcinol and Vanillin, while Coumarin was limit in *M. spicata* crude extract only.

Key word: Natural products, Phenolic compounds, HPLC, Iraqi medicinal herbs, Mantha

Introduction

Natural products continue to be one of the most important sources of lead compounds for the pharmaceutical industry (1). Plants synthesize a vast range of organic compounds that are traditionally classified as primary and secondary metabolites although the precise boundaries between the two groups can in some instances be somewhat blurred. Primary metabolites are compounds that have essential roles associated with photosynthesis, respiration, and growth and development. These include phytosterols, acyl lipids, nucleotides, amino acids and organic acids. Other phytochemicals, many of which accumulate in surprisingly high concentrations in some species, are referred to as secondary metabolites. These are structurally diverse and many are distributed among a very limited number of species within the plant kingdom and so can be diagnostic in chemotaxonomic studies. (2).

Natural products produced by plants has been estimated to be over 500,000 (3), and 160,000 of them have been identified, however natural products have played a significant role in human disease therapy and prevention (4). More than 60% and 75% of the chemotherapeutic drugs for cancer and infectious disease, respectively, are of natural origin (5) .

The largest class of natural products are terpenoids, also referred to as terpenes with more than 23,000 known compounds, and many of them have biological

*Corresponding Author:
Fathi A. Al-Mandeel
Lecturer,
Environmental and Pollution Control Research Center,
University of Mosul, Iraq.
E-mail: fathimandeel@yahoo.com

activities and are used for medical purposes. For example, the antimalarial drug Artemisinin and the anticancer drug paclitaxel (Taxol) are two of a few terpenes with established medical applications. (1).

The most well-known producers of active compounds are herbs such as the mint (Mentha) families, which is a member of the Lamiaceae (Labiatae), in the Order Lamiales, which includes many other families, such as Verbenaceae, Scrophulariaceae, and Acanthaceae. (6).

In Iraq Lamiaceae contain about 32 genus include Mentha and 140 species (7). Mentha is a genus of aromatic perennial herbs. It is distributed mostly in the temperate and sub-temperate regions of the world. Several Mentha species are considered industrial crops as they are a source of essential oils enriched in certain monoterpenes, widely used in food, flavour, cosmetic and pharmaceutical industries (8). This genus contains flavonoids, caffeic acid derivatives and essential oil (menthol or carvone as major compounds).

Because of the importance of many active compounds which are formed during the secondary metabolism pathways in Mentha species, the current study aimed to isolate some phenolic compounds from flowers extracts of *M. longifolia* and *M. spicata*

Materials And Methods

Five of standard phenolic compounds used to achieve the aims of the study by a series steps are:

A- Extraction Method

After drying the samples, extraction process completed by 95% ethanol, and ethanolic solution concentrating by use a rotary evaporator (RVE), then HCL (1N) used through acid Hydrolysis process for crude solution, and phenolic compounds isolated by Ethyl Acetate (9).

B- Methods Of Separation

After purification of crude extract by (0.1μM), filters membrane samples examined by ShimadZo, LC-2010 AHT (HPLC) which included C18(240 × 4.60mm) column chromatography which used for this processes, with 85% Acetonitril : 15% H_2O as mobile phase, 1mL/min rate of flow and detection at λ = 320nm wavelength,. Separation process was carried in the laboratories of Agriculture college / University of Mosul. Based on the principle that mentioned from (10).

C- Phenolic Compounds Standard

Standard of (Hydroquinone, Resorcinol, Coumarin, Vanillin and Gallic acid) were purchased from the Chemistry Department of Education College in Mosul University, one product of the Swiss Fluka company as well as BDH Co. Standard solution was prepared by dissolving 0.1 g of the compound in 10 ml of ethanol.

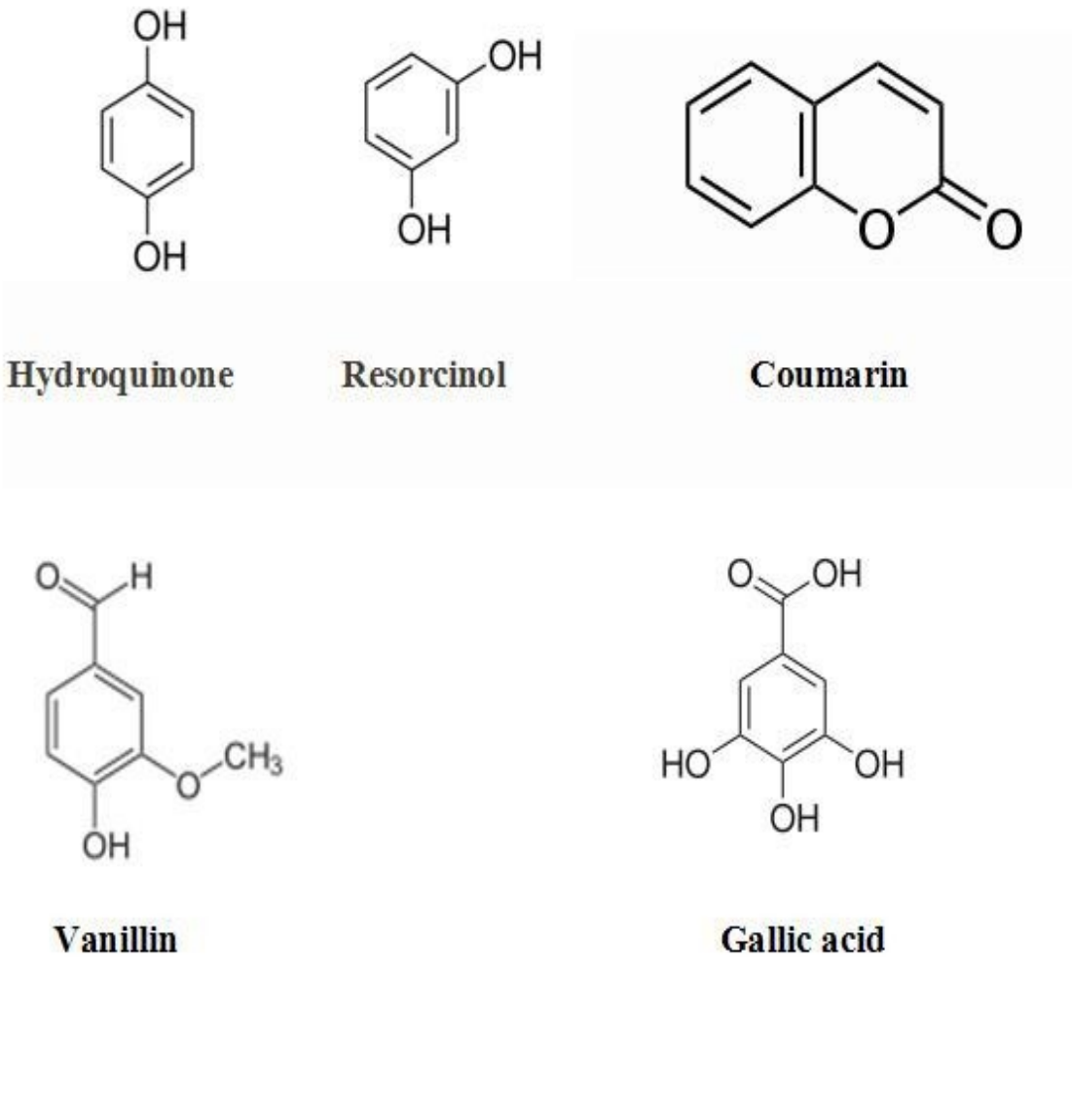
Results and Discussion

Phenolic compounds, often referred to by the generic term "phenolics", are a diverse and abundant group of naturally occurring plant substances. They are characterised by the possession of a hydoxylated aromatic nucleus and thus the term "phenolic" embraces several major classes of compound as well as occasional members of other groups that contain phenolic units. (11). The phenols of plants are one of the secondary metabolites classes that clearly characterized as aromatic metabolites which are vibrate from simple phenols such as Hydroquinone to complex phenols e.g. Subrine and Lignin.

In this study, five of phenolic compounds (Fig. 1) were separated after preparing extract crude of flowers that belonging to the *M. longifolia* and *M. spicata*, and the results of study showed that the tow crude extracts were similar by contains of Hydroquinone and Gallic acid

Published online in http://ijam.co.in
ISSN: 0976-5921
International Journal of Ayurvedic Medicine, 2013, 4(4), 352-360

while those extracts differed in their content of other phenolic compounds, so *M. longifolia* extract distinguished by presence of Resorcinol and Vanillin but Coumarin was limited in *M. spicata* extract only.



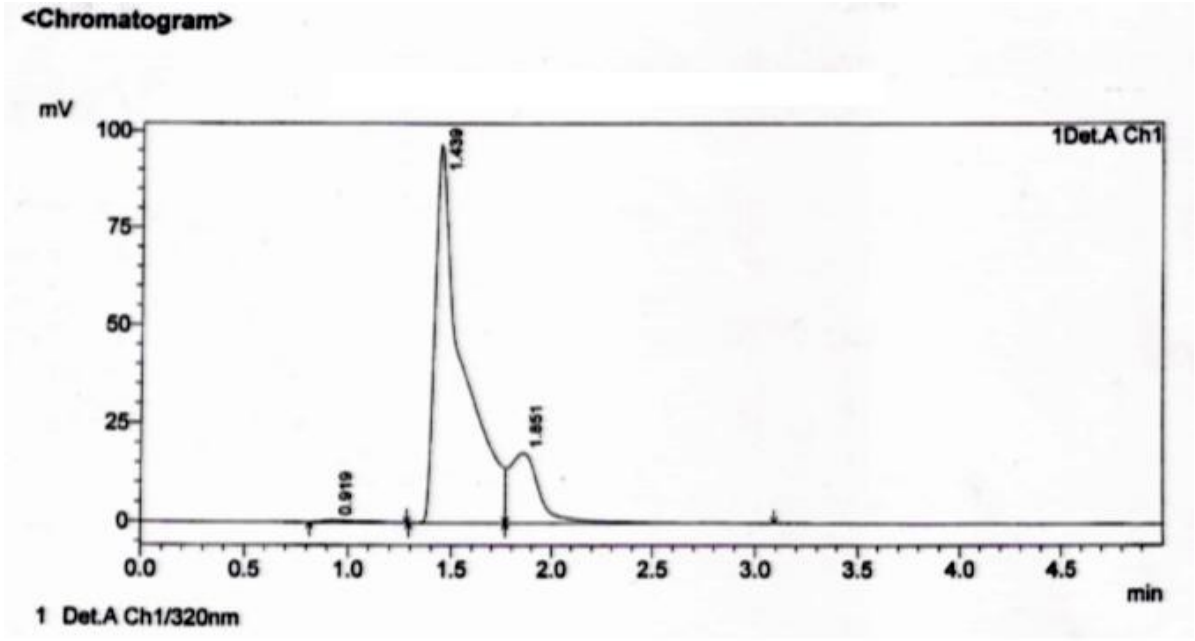
(Fig. 1) Formula of the phenolic compounds that have been separated in the current study.

Separation process led to draw of the curves of phenolic standard compounds (Fig. 2, 3, 4, 5 and 6) and the retention time which appearance on the Chromatogram was agreement with molecular weights of those standard, so the compounds that have high molecular weight took a longer time during the separation process compared to other compounds (Table 1), and then retention time (R_t) of standard adopted as conductor of their presence in the studied extracts. On this basis, the curves that appeared at 1.407 and 1.408 minutes (Table, 1) and (Fig., 7 and 8) refer to the Hydroquinone because those values identical to retention time 1.439 min. of Hydroquinone standrad (Fig. 2).

(Table. 1) Molecular weight and retention time of avtive compounds under study.

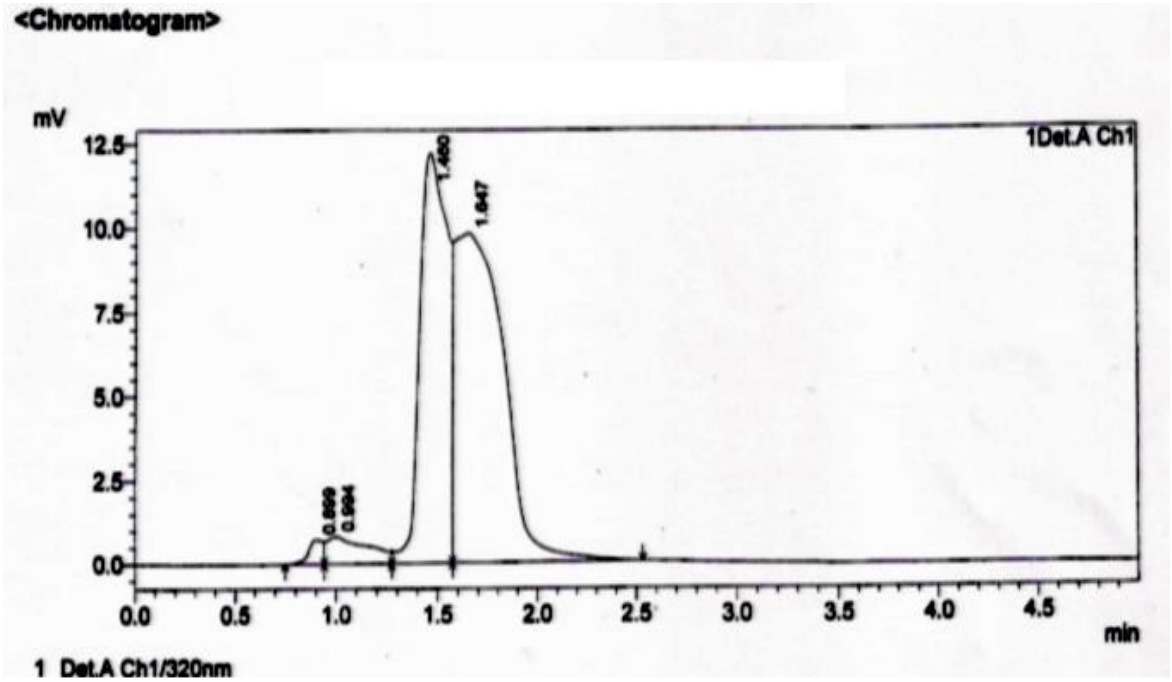
Standards			**Retention time(R_t) of compounds under study in crude.**	
Compounds	**Molcular weight**	**Retention time**	***M. spicata* L.**	***M. longifolia* L.**
Hydroquinone	**110.11**	**1.439**	**1.407**	**1.408**
Resorcinol	**110.11**	**1.647**	*****	**1.668**
Coumarin	**146.15**	**1.719**	**1.714**	*****
Vanillin	**152.15**	**1.743**	*****	**1.736**
Gallic acid	**170.12**	**2.430**	**2.389**	**2.365**

The results of separation process showed that the Resorcinol was found in extract flowers of *M. longifolia* , it was identified through the retention time of 1.668 min. (Fig. 3), while it was absent from the extract of *M. spicata*, This result was accord to Vanillin that observed at 1.736 min. But reversal got exactly for phenolic acid Coumarin which appeared in chromatogram of *M. spicata* only, at 1.714 min. retention time (Fig. 4 and 5).

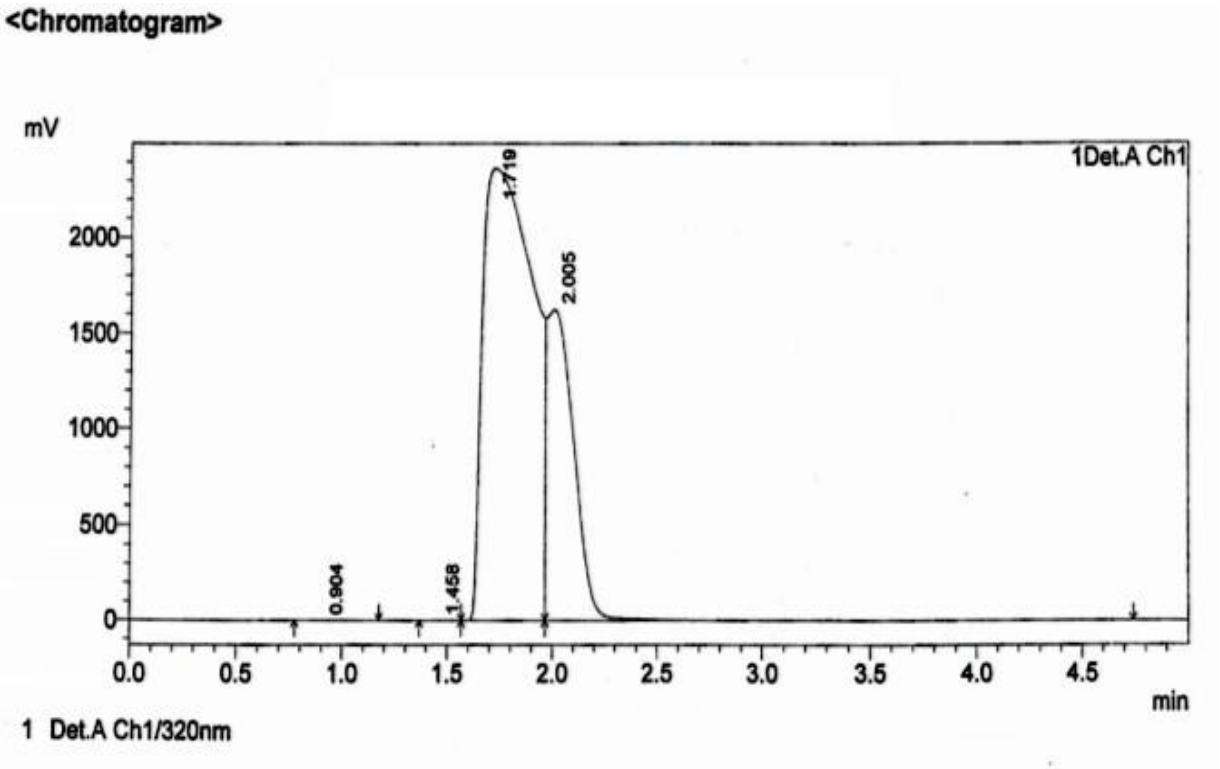


(Fig. 2) Chromatogram of Hydroquinone Standard, that appeared at 1.439 minutes by use Acetonitrile – Water, 85 : 15 v/v mobile phase in HPLC technique.

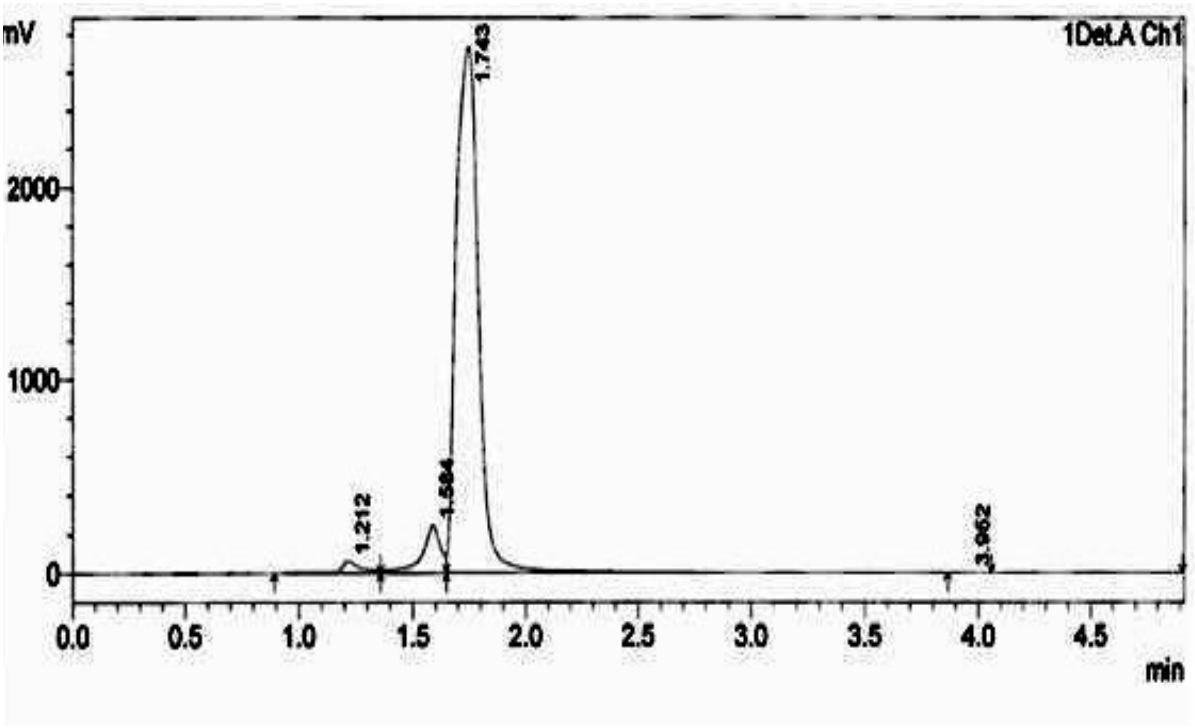
Published online in http://ijam.co.in
ISSN: 0976-5921
International Journal of Ayurvedic Medicine, 2013, 4(4), 352-360



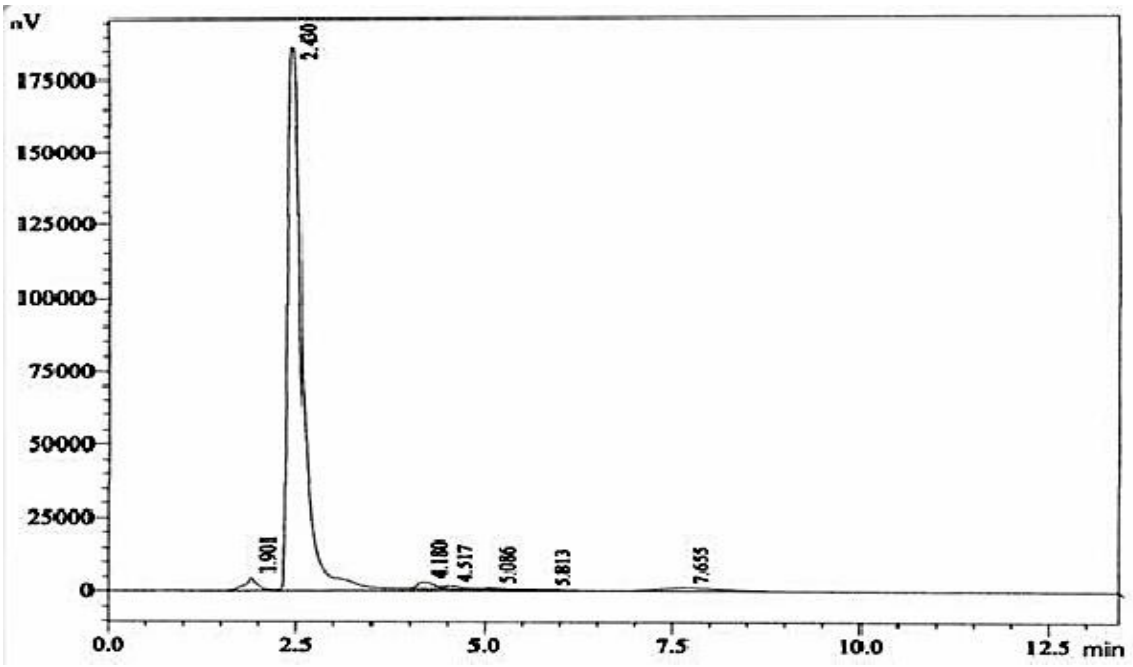
(Fig. 3) Chromatogram of Resorcinol Standard, that appeared at 1.647 minutes by use Acetonitrile – Water, 85 : 15 v/v mobile phase in HPLC technique.



(Fig. 4) Chromatogram of Coumarin Standard, that appeared at 1.719 minutes by use Acetonitrile – Water, 85 : 15 v/v mobile phase in HPLC technique.

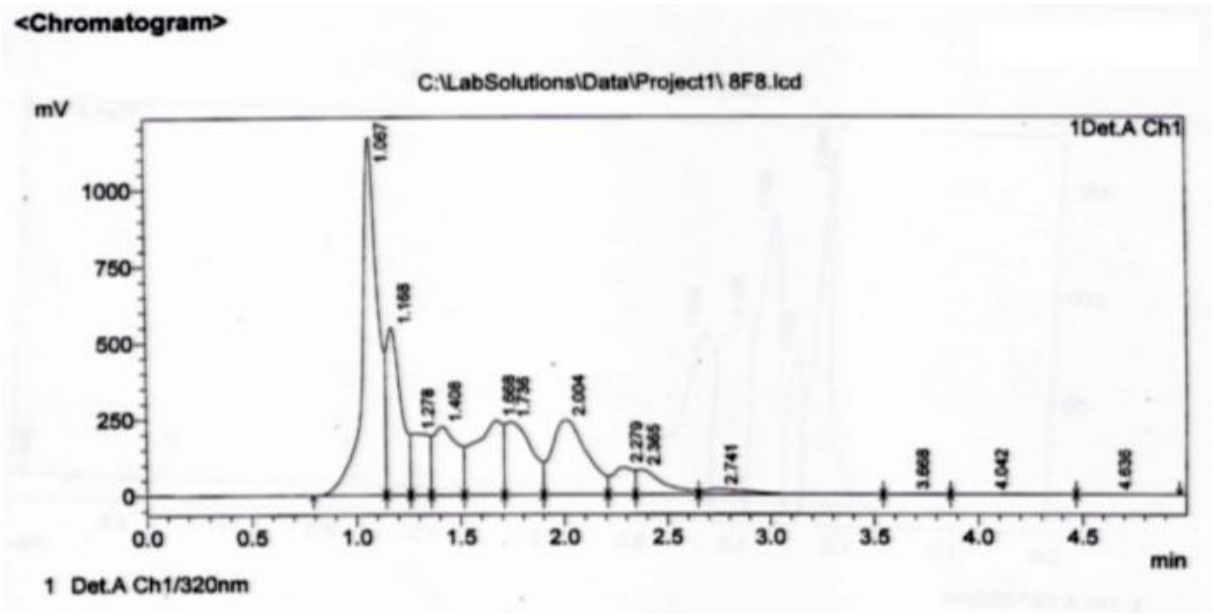


(Fig. 5) Chromatogram of Vanillin Standard, that appeared at 1.743 minutes by use Acetonitrile – Water, 85 : 15 v/v mobile phase in HPLC technique.

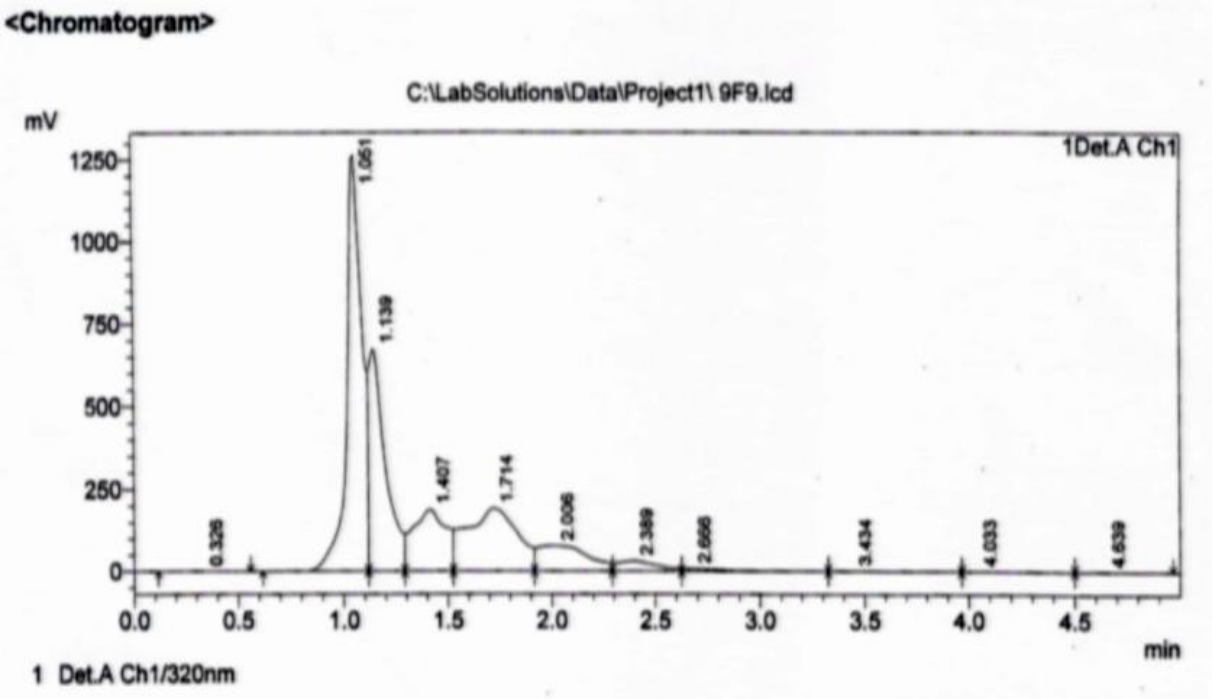


(Fig. 6) Chromatogram of Gallic acid Standard, that appeared at 2.430 minutes by use Acetonitrile – Water, 85 : 15 v/v mobile phase in HPLC technique.

Published online in http://ijam.co.in
ISSN: 0976-5921
International Journal of Ayurvedic Medicine, 2013, 4(4), 352-360



(Fig. 7) Chromatogram of M. longifolia extract crude by use Acetonitrile – Water, 85 : 15 v/v mobile phase in HPLC technique.



(Fig. 8) Chromatogram of M. spicata extract crude by use Acetonitrile – Water, 85 : 15 v/v mobile phase in HPLC technique.

Coumarins comprise a very large class of compounds found throughout the plant kingdom (12), (13). They are found at high levels in some essential oils, particularly cinnamon bark oil (7,000 ppm), cassia leaf oil (up to 87,300 ppm) and lavender oil. Coumarin is also found in fruits (e.g. bilberry, cloudberry), green tea and other foods such as chicory (14). Most coumarins occur in higher plants, with the richest sources being the Rutaceae and Umbelliferae. Although distributed throughout all parts of the plant, the coumarins occur at the highest levels in the fruits, followed by the roots, stems and leaves. Environmental conditions and seasonal changes can influence the occurrence in diverse parts of the plant (15).

With regard to the Gallic acid, it exists in plant material in the form of free acids, esters, catechin derivatives and hydrolysable tannins and Hydrolysis of tannic acid results in the release of glucose and gallic acid, which is a compound of great interest to both pharmaceutical and chemical industries Gallic acid is used in the manufacture of antimalarial drug Trimethoprim. It is also used to manufacture an antioxidant used in food industry (16).

In this study separation and identification of the Gallic acid, result to observed it in both plant extracts crude that (Fig. 7 and 8), so the retention time 2.365 and 2.389 min. were very close to the retention time 2.430 min. of Gallic acid standard (Fig. 6), which refers to the containment of these extracts on a Gallic acid.

For the ability to produce active compounds in plants that belonging to the genus *Mentha*, there is an abundance of studies carried out in this field and discussed various compounds that are synthesized through metabolic pathways for various species of *Mentha* and many active compounds were identified, including a phenolic group such as Luteolin, Hesperidin, Apigenin, Rosmarinic acid, Gardinin, which separated from the ethanolic extract of *M. piperita* by using of HPLC technique (17).In the latest study achieved in Mosul university by using analysis of HPLC on ethanolic extract of the dry leaves for two species of mint (*M. piperita* and *M. spicata*), (18) could separating and identifying a number of phenolic compounds include on a salicylic acid, Vanillin, p-hydroxy benzoic acid, Gallic acid, Catechol, Resorcinol, phenol and Quercetin.

References

1. Zhang, L. and Demain, A. L. Natural Products: Drug Discovery and Therapeutic Medicine. Totowa, New Jersey; Humana Press Inc; 2005. 382 pages.
2. Crozier, A., Clifford, M. N. and Ashihara, H. Plant Secondary Metabolites Occurrence, Structure and Role in the Human Diet. by Blackwell Publishing Ltd, 2006; 372 pages.
3. Mendelson, R., Balick, M.J. The value of undiscovered pharmaceuticals in tropical forests. Econ Bot; 1995. 49:223–228
4. Newman, D.; Cragg, G. and Snader, K. The influence of natural products upon drug discovery. Journal of Natural Product Reports; 2000. 17(3):215–234.
5. Newman D., Cragg, G. and Snader K. Natural products as sources of new drugs over the period 1981–2002. Journal of Natural Products; 2003. 66(7):1022–1037
6. Lawrence, B.M. Mint: The Genus Mentha (Medicinal and Aromatic Plants - Industrial Profiles); London; Taylor & Francis Group, LLC; 2007. 576 pages.
7. Al-Moussawi, H.A. Plant Txonomy. University of Baghdad, Ministry of Higher Education and scientific Research. 1987. 241 pages.
8. Bhat, S.; Maheshwari, P.; Kumar, S. and Kumar, A. Mentha species: In vitro Regeneration and genetic transformation.

Journal of Molecular Biology Today ; 2002. 3(1):11-23.
9. Harborne, J.B. Phytochemical Methods: A Guide to Modern Technique of Plant Analysis. 1st ed., London. Cox and Wyman; 1973. 278 pages.
10. Guedon, D. J. and Pasquier, B. P. Analysis and distribution of flavonoid glycosides and rosemarinic acid in 40 Mentha x piperita clones. Journal of Agriculture Food Chemistry; 1994. 42:679-634.
11. Robinson T. The Organic Constituents of Higher Plants" 4th Edition Their chemistry and interrelationships; Cordus Press. Mass.,U.S.A; 1980. 352 pages.
12. Egan, D.; O'Kennedy, R.; Moran, E.; Cox, D.; Prosser, E. and Thornes, R.D. The pharmacology, metabolism, analysis and applications of coumarin and coumarin-related compounds. Journal of Drug Metab Rev; 1990. 22: 503-529.
13. Finn, G.; Kenealy, E.; Creaven, B. and Egan, D. In vitro cytotoxic potential and mechanism of action of selected coumarins, using human renal cell lines. Journal of Cancer Letts; 2002. 183: 61-68.
14. Lake, B. Coumarin metabolism, toxicity and carcinogenicity: relevance for human risk assessment. Journal of Food and Chemical Toxicology; 1999. 37: 423-453.
15. Keating, G. and O'Kennedy R. The chemistry and occurrence of coumarins. coumarins: biology, applications and mode of action. (Eds: O'Kennedy R. and Thornes, R. D.). Chichester, John Wiley & Sons; 1997 : 23-66.
16. Beena, P.S.; Basheer, S.; Bhat, S.G.; Bahkali, A.H. and Chandrasekaran, M. Propyl gallate synthesis using acidophilic tannase and simultaneous production of tannase and gallic acid by marine Aspergillus awamori BTMFW032. Journal of Applied Biochemistry and Biotechnology ; 2011 . 164;612–628.
17. Areias, F. M.; Valentao, P.; Andrade, P. B.; Ferreres, F. and Seabra, R. M. Phenolic fingerprint of peppermint leaves. Journal of Food Chemistry; 2001. 73(3); 307-311.
18. Al-Hashimy, F. H. Effect of Nitrogen Fertilization, Spraying of Gibberellin, Algamix and Harvest Date on Growth, Production and Identification of the Active Constituents of Two Mentha sp. Ph.D. Thesis, Mosul Univ. College of Agriculture; 2012. 251 pages

*****