International Journal of Pharmacy and Chemistry
2023; 9(1): 1-8
http://www.sciencepublishinggroup.com/j/ijpc
doi: 10.11648/j.ijpc.20230901.11
ISSN: 2575-5730 (Print); ISSN: 2575-5749 (Online)

Determination of Antioxidant Capacity and HPLC Analysis of Gallic Acid Plus Rutin in Some Lamiaceae Plants Growing in the East of Libya

Abdullah Alamami[1], Samar Ben Zaed[2], Esra El Naili[3], Areej Daboub[2], Naema Elremali[3], Fatma Elshibani[4, *], Salim Skaik[5]

[1]Department of Basic Medical Science, Faculty of Pharmacy, University of Benghazi, Benghazi, Libya

[2]Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmacy, University of Zawia, Tripoli, Libya

[3]Department of Chemistry, Faculty of Pharmacy, University of Benghazi, Benghazi, Libya

[4]Department of Pharmacognosy, Faculty of Pharmacy, University of Benghazi, Benghazi, Libya

[5]Department of Pharmacognosy, Faculty of Pharmacy, Assalam International University, Benghazi, Libya

Email address:

fatma.elshibani@uob.edu.ly (Fatma Elshibani)

[*]Corresponding author

To cite this article:

Abdullah Alamami, Samar Ben Zaed, Esra El Naili, Areej Daboub, Naema Elremali, Fatma Elshibani, Salim Skaik. Determination of Antioxidant Capacity and HPLC Analysis of Gallic Acid Plus Rutin in Some Lamiaceae Plants Growing in the East of Libya. *International Journal of Pharmacy and Chemistry*. Vol. 9, No. 1, 2023, pp. 1-8. doi: 10.11648/j.ijpc.20230901.11

Received: November 17, 2022; **Accepted**: February 4, 2023; **Published**: February 27, 2023

Abstract: Lamiaceae family comprise a wide range of medicinal and aromatic plant species with strong antioxidant properties and multiple pharmaceutical applications in folk medicine. *Mentha piperita* L., *Ocimum basilicum*, *Origanum vulgare* L, *Rosmarinus officinalis* L, *Salvia officinalis* L. and *Thymus capitatus,* are edible plants in Libya, belonging to this family. The current work aims to evaluate gallic acid and rutin contents of the methanolic extract of the mentioned plants using RP- High performance liquid chromatography (HPLC) and assess their antioxidant properties by applying the DPPH radical scavenging assay. The content of gallic acid were found to be considerable in all plants studied, most remarkably in *Rosmarinus officinalis* L. and *Thymus capitatus* with area under the peak (56.14% and 54.62% respectively), while lower contents were detected in *Ocimum basilicum* (34.44%), *Origanum vulgare* L. (28.98%), *Mentha piperita* L.(26.09%), and *Salvia officinalis* L. (24.34%). On the other hand, it was noted that rutin is found in less quantities compared to previous results. The extracts of *Organium vulgare* and *Thymus capitatus* had a significant antioxidant potential compared to standard vitamin C with 0.34 ± 0.03 and 0.46 ± 0.02 mg/mL respectively, while a lower scavenging capacity were recoded for *Mentha piperita* L. (0.59 mg/mL ± 0.01), *Salvia officinalis* L. (0.60 mg/mL ± 0.01), *Rosmarinus officinalis* L (0.65 mg/mL ± 0.07), and *Ocimum basilicum* (0.99 mg/mL ± 0.02). In conclusion, the reported results point out the significance of this family as a source of antioxidant agents with high promising capability to affect the redox state.

Keywords: Libya, Gallic Acid, Rutin, DPPH, HPLC

1. Introduction

Secondary plant metabolites consist of a diverse range of medicinally active complex compounds, including flavonoids, phenolic acids, tannins, and lignans. These chemicals have significant benefits against a variety of long-term illnesses, including cancer, aging, diabetes, and degenerative disorders [1]. Frequent exposure to oxidative stress radicles may result in DNA and protein mutations, as well as lipid damage, which may lead to cancer. Since natural polyphenolic chemicals have higher antioxidant potency, there is increased interest in isolating and using them as antioxidants. According to the structure of this class of chemicals, they can be employed to reduce oxidative damage by serving as a scavenger of free radicals, singlet oxygen, and metal ions [2]. As a result, the researchers focused their

efforts on polyphenols, which possess the potential to improve human health.

It is well known that, the Lamiaceae plant family is abundant in the most common secondary metabolites, such as flavonoids and phenolics [3] as it involves around 7000 species and 245 genera [4]. The species in this family are distinguished by their square stems and opposing leaves. The flowers are also bisexual and zygomorphic. The majority of the plants in this family contain essential oil a prominent component [5]. The aromatic volatile oils are concentrated in the plant's aerial parts, particularly the leaves. The extracted oil can be used to produce flavors, cosmetics and perfumes, as well as in the manufacture of insecticides, and pharmaceutical products [6].

Some species belonging to Lamiaceae family are famous culinary herbs in Africa such as basil (*Ocimum basilicum*.), thyme (*Thymus capitatus*), oregano (*Origanum vulgare* L.), rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.), and Mint (*Mentha piperita* L.). The therapeutic activity of these herbs is specified in the traditional medicine system as a remedy for various disorders [7-9].

The essential oils and phenolic compounds were isolated The phenolic compounds and essential oils derived from these plants are of particular interest in pharmacology. There are various published studies regarding the efficacy of the investigated plants as antioxidant, anti-inflammatory, antimicrobial agents, or sedatives *etc* [8, 9]. The active constituents, traditional applications, and pharmacological activity of these herbs were summarized in (Tables 1 and 2).

Table 1. *Data on the study of the composition of the essential oil, phenolics and flavonoids of medicinal plant raw materials from the Lamiaceae family.*

Medicinal plant	Essential oil	Phenolics and flvonoids
M. piperta	Menthone, carvon, menthol, menthyl acetate, menthofur, germacrene-D, 1,8-cineole, neo-menthol, β-Pinene, trans-caryophyllene, α-Pinene, neoisomenthyl acetate, and trans-sabinene hydrate. [10-13]	Quercetin, luteolin and rosmarinic acid [14], eriocitrin, narirutin, hesperidin, isorhoifolin, diosmin, luteolin-7-O-rutinoside, caffeic and rosmarinic acid, and 5,7-dihydroxycromone-7-O-rutinoside [15, 16], chlorogenic acid and rutin [17, 18].
O. bacsilicum	Chavicol methyl ether (estragol), linalool, eugenol, 1, 8- cineole, methyl cinnamate and linalool [19, 20].	Caftaric, caffeic, gentisic, *p*-coumaric, ferulic and chlorogenic acids), (rutin, quercitin and isoquercitrin), and free flavonoid aglycons (luteolin, quercetin) [21].
O. vulgare L	Carvacrol, thymol, linalyl acetate, (*Z*)-α-bisabolene, (*E*)-*β*-caryophyllene, and caryophyllene oxide, linalool, terpinen-4-ol, and sabinene hydrate [22-24].	Protocatechuic acid ester derivatives, origanol A and origanol B [25], Acacetin and apegenin dervatives [26].
R. officinalis	1,8-Cineol, α-pinene, camphor, borneol and camphene [27-31].	Carnosic acid, carnosol and rosmarinic acid [32], luteolin, apigenin, hispidulin and a dihydroxy-dimethoxyflavone [33].
S. officinalis	1,8-Cineole, camphor, *α*-thujone, *β*-thujone, borneol, viridiflorol, α-pinene, β-pinene, camphene, β-myrcene [34, 35].	Ellagic acid and chlorogenic acid, [34-36], epigallocatechin gallate, epicatecin, rosmarinic acid, quercetin, rutin and luteolin-7-glucoside [34], p-hydroxybenzoic acid, 3-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, syringic acid, caffeic acid, p-coumaric acid, rosmarinic acid, vanillic acid, ferulic acid), and flavonoids (kaempferol, chrysin, galangin, myricetin, luteolin and pinocembrin) [36].
T. capitatus	Thymol, Carvacrol, γ-terpinene, p-cymene, linalool and β-caryophyllene [31, 37-39].	Tannic acid, gallic acid, chlorogenic hemihydrate, caffeic acid, syringic acid as well as ferulic acid, *p*-coumaric acid, and *trans*-cinnamic and rosmarinic acids and (flavonoids quercetin, luteolin, and apigenin) [40, 41].

Table 2. *Data on the study of traditional uses and pharmacological activities of medicinal plant raw materials from the Lamiaceae family.*

Medicinal plant	Traditional uses	Pharmacological activity
M. piperta	Antimicrobial, sedative, analgesic, carminative [42].	Antiallergic [15], antimicrobial [43], antioxidant [44].
O. bacsilicum	kidney problems, as a hemostatic in childbirth, earache, menstrual irregularities, arthritis, anorexia, treatment of colds and malaria [45].	Antibacterial, antioxidant, antiviral, antibacterial and antifungal [46-48].
O. vulgare L	Treatment of cold, cough, and digestive disorders [49].	Antimicrobial and antioxidant activities [25, 50], anti-ulcer, anti-inflammatory, antidiabetic, antiviral, cytotoxic and antitumour [26].
R. officinalis	Antimicrobial, antithrombic, diuretic, antidiabetic and hepatoprotective [43, 51-56].	Antimicrobial [43, 51], antimutagenic, antioxidant anti-inflammatory, antiseptic, antispasmodic, anti-diabetic, anti-ulcerogenic, hepatoprotective and antidepressant [56-60].
S. officinalis	Treatment of seizure, ulcers, gout, inflammation, diabetis, dizziness, tremor, paralysis and diarrhea [34].	Antimicrobial [34, 51, 52, 61], antioxidant [35, 61, 62], antidiabetic, anti-obesity, gastroprotective, antispasmatic, anti-inflammatory, virucidal, fungicidal, and bactericidal [63], antimutagenic, antidementia, hypoglycemic, and hypolipidemic effects [34].
T. capitatus	Anthelmintic, carminative, antispasmodic, emmenagogue, rubefactient, sedative, expectorant, tonic and stimulant [66].	Antimicrobial, antioxidant cytotoxic, antinociceptive and hypoglycemic activities [25, 64-67].

Since there are so many phenolic compounds in plants, quantifying and identifying each phenolic acid and flavonoid is a time-consuming task [68]. Different techniques have been used for the separation and quantification of these compounds, while thin layer chromatography (TLC) and high-performance liquid chromatographic (HPLC) being the most used.

The goal of this study was to estimate the rutin and galic acid contents, as well as the antioxidant intensity of cold methanolic extracts of mint, basil, oregano, rosmary, sage, and thyme aerial parts, using the radical scavenging activity assay on DPPH (2,2-diphenyl-1-picrylhydrazyl), and to compare these data findings with other published data.

2. Material and Methods

2.1. Plant Material

The aerial parts of the studied plants were collected from Aljabal Al-khathar, about 200 km east of Benghazi/Libya during February.

The department of Pharmacognosy (Faculty of Pharmacy, University of Benghazi) verified the plant species. The collected plants were left to dry in the open air, then were grinded by the blender into course powder and kept to be used for extraction.

2.2. Extraction and Sample Preparation

About 50 gm of the powder was extracted with methanol 70% at room temperature. After filtration, the extract was concentrated by removing under a vacuum at 40°C. The obtained crude extracts were then kept in dark at 4°C for phytochemical screening, chromatographic analysis and antioxidant studies.

2.3. High Performance Liquid Chromatography (HPLC)

HPLC described method by Nouraei et al. (2018) [69] was used with some modifications for separation and identification of rutin and gallic acid in the prepared samples. Reversed phase-high performance chromatography (RP-HPLC) was adopted for fingerprinting of the methanol extracts of the aerial part of the plants based on phenolic composition. The crude extract volume was adjusted to 100 mL in a volumetric flask. Twenty µL of each sample were analyzed for phenolics using a Jasco LC-Net/APC HPLC system equipped with Jasco PU-2080 plus pump and Jasco UV-2070 plus UV detector. Separation was achieved using a INertsil ODS-3 C18 column (particle size 5 µm, 250 mm × 4.6 mm∅). An isocratic elution mode was adopted using 0.1% (v/v) phosphoric acid in water: acetonitrile 80: 20 (v/v), at a flow rate of 1.5 mL/min and eluted compounds were detected at a wavelength of 310 nm. Each sample was injected in triplicate; the acquired data were processed and the peaks integrated using Chrom NAV software.

2.4. Antioxidant Activity

Free radical scarving capacity of the plant extract samples were estimated according to previously reported method (Gul et al., 2016) with slight modification [70], which depending on the change of the intensity of the purple-colored DPPH (2,2- diphenylpicrylhydrazyl). In brief, for antioxidant activity measurement, the different concentrations of the plant extract (50, 100, 300, and 500 mg/L) were prepared by the dilution with pure methanol.

A solution of DPPH (0.1 mM, 5 mL) was mixed with 100 µL of the diluted extracts. All the mixtures were shaken vigorously and kept to stand at room temperature for 30 min in the dark for any reaction to take place. Absorbance of the reaction mixture was measured at 517 nm spectrophotometrically using a spectrophotometric device (Hitachi F-2500). Absorbance of the DPPH with methanol solution was also measured as a negative control, while BHT was employed as the positive compound for comparison. All the determinations were performed in triplicate.

The capability to scavenge the DPPH radical was calculated as a percentage of free radical inhibition (I %) according to this formula:

$$I\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

Where; A_{blank} is absorbance of the control reaction at t=0 min, and A_{sample} is the absorbance of the extract at t=30 min. The values of IC50 were calculated by plotting the graph of extract concentrations against the scavenging activity. Finally, radical scavenging activity was expressed as IC50 (µg ml^{-1}), the antiradical dose required to cause a 50 % inhibition.

3. Results

The results of gallic acid and rutin content and the antioxidant activities of the methanolic extracts of the tested plants are illustrated in Tables 3 and 4.

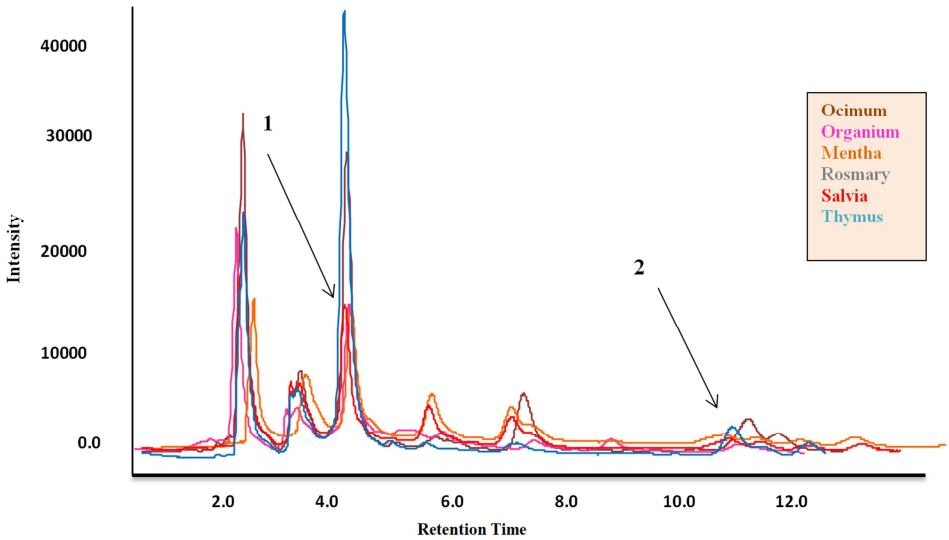

Figure 1. *RP-HPLC chromatogram of the methanol extracts of the plants for investigation of Gallic acid and Rutin.*

Table 3. *Chromatographic data of major peaks detected via RP-HPLC analysis of the methanol extracts of the aerial parts of the sex investigated plants.*

Peak	R_t	Area under the peak (%) in the different plants					
		Ocimum	*Organium*	*Mentha*	*Rosmary*	*Salvia*	*Thymus*
1	2.370	37.1	30.33	20.91	25.28	24.35	26.10
2	3.535	12.28	10.66	10.84	16.58	10.21	10.81
3	4.255*	34.44	28.98	26.09	56.14	24.34	54.62
4	5.422	0	0.02	12.87	4.22	8.68	0.08
5	7.022	9.66	0.04	6.96	6.67	4.15	0.03
6	11.455**	8.99	2.73	1.34	3.68	3.25	4.35

*Corresponding to standard gallic acid, while **Corresponding to standard rutin

Table 4. *The antioxidant activity of the plants under investigation Against vitamin C. terms of $IC_{50} \pm SD$.*

plant	DPPH IC50 (mg/mL)
Ocimum basilicum	0.99 ± 0.02
Organium vulgare	0.34 ± 0.03
Mentha piperta	0.59 ± 0.01
Rosmarinus officinalis	0.65 ± 0.07
Salvia officinalis	0.60 ± 0.01
Thymus capitatus	0.46 ± 0.02
Vitamin c	0.28 ± 0.002

4. Discussion

An obvious variability in the composition was observed upon examination of the overlay of the HPLC chromatograms of the analyzed samples. Peaks no 2 and 6 (of respective R_t values 4.255 and 11.455) were identified as gallic acid and rutin, through comparison with authentic samples run under the same experimental conditions confirming, in this respect, the results obtained by TLC. Although. In fact, based on peak area measurements, a component equivalent to gallic acid were found to be abundant in all plants studied, most notably in rosmary and thymus with area under the peak (56.14 and 54.62 respectively). Researchers who have worked with thyme and rosemary reported remarkable gallic acid concentrations in both plants [71, 40]. Earlier reports displayed that basil, oregano and sage extracts comprised appreciable quantities of gallic acid [72-75]. Other data showed that the fresh rosemary, thyme, sage, oregano, mint and basil contained a relatively high average quantity of rosmarenic acid and law amount of gallic acid [76]. However, it is notable that the extracts of thyme and sage lack of gallic acid [77], this reported study was to some extent different from the detected results, may be attributed to the method of preparation.

On the other hand, it was noted that rutin is found in the mentioned plants in small quantities. These results are similar to those reported for *oregano* and *sage* [34, 74, 78, 79], meanwhile, rutin was appeared to predominate in the ocimum [21, 80], as well as in rosmaey [79]. Accordingly, comparing to values in literature [81], rutin was relatively low in the methanolic extract of mint. The recent study showed that the recoveries of rutin found in thyme, sage and rosmary, that growing in Libya were fluctuate which may be due to various agricultural environments [82].

The results for IC50 values were ranged from (0.34 ± 0.03 to 0.99 ± 0.02) µg/ml, the methanolic extract of oregano and thyme when compared to vitamin C, showed a remarkable scavenging potency on the DPPH radical (0.34 and 0.46 µg/ml respectively). Obviously, these results agree with the literature data described by [17, 74, 82, 83], which displayed that the oregano, mint, sage, rosemary and thyme extracts have a potent radical scavenging activity.

In general, all the tested plants exhibited an antioxidant property with different powers; this result strongly suggests that these extracts contain the necessary compounds to reduce oxidative stress in human beings.

In fact, it has been found that antioxidant molecules such as polyphenols, flavonoids, and tannins are responsible for the non-enzymatic antioxidant activity for these plants, which can be observed by reducing and discoloring of DPPH, this activity due to their hydrogen donating ability [84].

5. Conclusion

Mentha piperta, Ocimum basilicum, Origanum vulgare, Rosmarinus officinalis, Salvia officinalis and *Thymus capitatus* are an important plants growing and cultivated in El-Jabal Al Akhdar (Libya) belong to (Lamiacea) family which is regarded as a potential source of important bioactive compounds.

Reported results indicate the importance of this family as a source of antioxidant compounds with high ability to affect the redox state of cells according to the disease type associated with oxidative stress. However, further *in-vivo* and *in-vitro* evaluations are required with other scientific investigations to understand the mechanism behind its significant medicinal importance in preventing various diseases induced by oxidative stress. Also, further analysis is needed to develop novel drugs for the management of oxidative stress and associated diseases.

References

[1] Droge W. Free radicals in the physiological control of cell function. Physiological reviews. 2002 Jan 1; 82 (1): 47-95.

[2] Wanasundara PK, Shahidi FJ. Antioxidants: science, technology, and applications. Bailey's industrial oil and fat products. 2005 Jul 15.

[3] Wink M. Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry. 2003 Sep 1; 64 (1): 3-19.

[4] Shanaida M, Golembiovska O, Hudz N, Wieczorek PP. Phenolic compounds of herbal infusions obtained from some species of the Lamiaceae family. Current Issues in Pharmacy and Medical Sciences. 2018 Dec 1; 31 (4): 194-9.

[5] Lawrence BM, Harley RM, Reynolds T. Chemical components of labiatae oils and their exploitation, pp: 399-436. Advances in Labiate science. Kew: Royal Botanic Gardens. UK. 1992.

[6] Özkan M. Glandular and eglandular hairs of Salvia recognita Fisch. & Mey. (Lamiaceae) in Turkey. Bangladesh Journal of Botany. 2008; 37 (1): 93-5.

[7] Ličina BZ, Stefanović OD, Vasić SM, Radojević ID, Dekić MS, Čomić LR. Biological activities of the extracts from wild growing Origanum vulgare L. Food control. 2013 Oct 1; 33 (2): 498-504.

[8] Carović-StanKo K, PeteK M, Grdiša M, Pintar J, Bedeković DA, Satovic Z. Medicinal plants of the family Lamiaceae as functional foods–a review. Czech journal of food sciences. 2016 Oct 31; 34 (5): 377-90.

[9] Milevskaya VV, Prasad S, Temerdashev ZA. Extraction and chromatographic determination of phenolic compounds from medicinal herbs in the Lamiaceae and Hypericaceae families: A review. Microchemical Journal. 2019 Mar 1; 145: 1036-49.

[10] Keawsa-ard S, Kongtaweelert S. Antioxidant, antibacterial, anticancer activities and chemical constituents of the essential oil from Mesua ferrea leaves. Chiang Mai J. Sci. 2012 Jul 1; 39 (3): 455-63.

[11] Saharkhiz MJ, Motamedi M, Zomorodian K, Pakshir K, Miri R, Hemyari K. Chemical composition, antifungal and antibiofilm activities of the essential oil of Mentha piperita L. International Scholarly Research Notices. 2012; 2012.

[12] Taherpour AA, Khaef S, Yari A, Nikeafshar S, Fathi M, Ghambari S. Chemical composition analysis of the essential oil of Mentha piperita L. from Kermanshah, Iran by hydrodistillation and HS/SPME methods. Journal of Analytical Science and Technology. 2017 Dec; 8: 1-6.

[13] Beigi M, Torki-Harchegani M, Ghasemi Pirbalouti A. Quantity and chemical composition of essential oil of peppermint (Mentha× piperita L.) leaves under different drying methods. International Journal of Food Properties. 2018 Jan 1; 21 (1): 267-76.

[14] Hadjmohammadi M, Karimiyan H, Sharifi V. Hollow fibre-based liquid phase microextraction combined with high-performance liquid chromatography for the analysis of flavonoids in Echinophora platyloba DC. and Mentha piperita. Food chemistry. 2013 Nov 15; 141 (2): 731-5.

[15] Inoue T, Sugimoto Y, Masuda H, Kamei C. Antiallergic effect of flavonoid glycosides obtained from Mentha piperita L. Biological and Pharmaceutical Bulletin. 2002; 25 (2): 256-9.

[16] Sroka Z, Fecka I, Cisowski W. Antiradical and anti-H2O2 properties of polyphenolic compounds from an aqueous peppermint extract. Zeitschrift für Naturforschung C. 2005 Dec 1; 60 (11-12): 826-32.

[17] Farnad N, Heidari R, Aslanipour B. Phenolic composition and comparison of antioxidant activity of alcoholic extracts of Peppermint (Mentha piperita). Journal of Food Measurement and Characterization. 2014 Jun; 8 (2): 113-21.

[18] Mišan A, Mimica-Dukić N, Mandić A, Sakač M, Milovanović I, Sedej I. Development of a rapid resolution HPLC method for the separation and determination of 17 phenolic compounds in crude plant extracts. Open Chemistry. 2011 Feb 1; 9 (1): 133-42.

[19] Adham AN. Comparative extraction methods, phytochemical constituents, fluorescence analysis and HPLC validation of rosmarinic acid content in Mentha piperita, Mentha longifolia and Osimum basilicum. J. Pharmacogn. Phytochem. 2015; 3 (6): 130-9.

[20] Hussain AI, Anwar F, Sherazi ST, Przybylski R. Chemical composition, antioxidant and antimicrobial activities of basil (Ocimum basilicum) essential oils depends on seasonal variations. Food chemistry. 2008 Jun 1; 108 (3): 986-95.

[21] Vlase L, Benedec D, Hanganu D, Damian G, Csillag I, Sevastre B, Mot AC, Silaghi-Dumitrescu R, Tilea I. Evaluation of antioxidant and antimicrobial activities and phenolic profile for Hyssopus officinalis, Ocimum basilicum and Teucrium chamaedrys. Molecules. 2014 May; 19 (5): 5490-507.

[22] Morshedloo MR, Salami SA, Nazeri V, Maggi F, Craker L. Essential oil profile of oregano (Origanum vulgare L.) populations grown under similar soil and climate conditions. Industrial Crops and Products. 2018 Sep 1; 119: 183-90.

[23] Mancini E, Camele I, Elshafie HS, De Martino L, Pellegrino C, Grulova D, De Feo V. Chemical composition and biological activity of the essential oil of Origanum vulgare ssp. hirtum from different areas in the Southern Apennines (Italy). Chemistry & biodiversity. 2014 Apr; 11 (4): 639-51.

[24] Rao GV, Mukhopadhyay T, Annamalai T, Radhakrishnan N, Sahoo MR. Chemical constituents and biological studies of Origanum vulgare Linn. Pharmacognosy research. 2011 Apr; 3 (2): 143.

[25] Faleiro L, Miguel G, Gomes S, Costa L, Venâncio F, Teixeira A, Figueiredo AC, Barroso JG, Pedro LG. Antibacterial and antioxidant activities of essential oils isolated from Thymbra capitata L. (Cav.) and Origanum vulgare L. Journal of Agricultural and Food Chemistry. 2005 Oct 19; 53 (21): 8162-8.

[26] Zhang XL, Guo YS, Wang CH, Li GQ, Xu JJ, Chung HY, Ye WC, Li YL, Wang GC. Phenolic compounds from Origanum vulgare and their antioxidant and antiviral activities. Food chemistry. 2014 Jun 1; 152: 300-6.

[27] Lahlou M, Berrada R. Composition and niticidal activity of essential oils of three chemotypes of Rosmarinus officinalis L. acclimatized in Morocco. Flavour and fragrance journal. 2003 Mar; 18 (2): 124-7.

[28] Touafek O, Nacer A, Kabouche A, Kabouche Z, Bruneau C. Chemical composition of the essential oil of Rosmarinus officinalis cultivated in the Algerian Sahara. Chemistry of Natural Compounds. 2004 Jan; 40 (1): 28-9.

[29] Marzouk Z, Neffati A, Marzouk B, Chraief I, Fathia K, Ghedira LC, Boukef K. Chemical composition and antibacterial and antimutagenic activity of Tunisian Rosmarinus officinalis L. oil from Kasrine. JOURNAL OF FOOD AGRICULTURE AND ENVIRONMENT. 2006 Jul; 4 (3/4): 61.

[30] Gachkar L, Yadegari D, Rezaei MB, Taghizadeh M, Astaneh SA, Rasooli I. Chemical and biological characteristics of Cuminum cyminum and Rosmarinus officinalis essential oils. Food chemistry. 2007 Jan 1; 102 (3): 898-904.

[31] El-Hawary, S., El-Shabrawy, A., Ezzat, S., & El-Shibany, F. Gas chromatography-mass spectrometry analysis, hepatoprotective and antioxidant activities of the essential oils of four Libyan herbs. Journal of Medicinal Plants Research, 2013.*7* (24), 1746-1753.

[32] Kontogianni VG, Tomic G, Nikolic I, Nerantzaki AA, Sayyad N, Stosic-Grujicic S, Stojanovic I, Gerothanassis IP, Tzakos AG. Phytochemical profile of Rosmarinus officinalis and Salvia officinalis extracts and correlation to their antioxidant and anti-proliferative activity. Food chemistry. 2013 Jan 1; 136 (1): 120-9.

[33] Linares, I. B., Arráez-Román, D., Herrero, M., Ibáñez, E., Segura-Carretero, A. and Fernández-Gutiérrez, A., 2011. Comparison of different extraction procedures for the comprehensive characterization of bioactive phenolic compounds in Rosmarinus officinalis by reversed-phase high-performance liquid chromatography with diode array detection coupled to electrospray time-of-flight mass spectrometry. *Journal of Chromatography A*, *1218* (42), pp. 7682-7690.

[34] Ghorbani A, Esmaeilizadeh M. Pharmacological properties of Salvia officinalis and its components. Journal of traditional and complementary medicine. 2017 Oct 1; 7 (4): 433-40.

[35] El Euch SK, Hassine DB, Cazaux S, Bouzouita N, Bouajila J. Salvia officinalis essential oil: Chemical analysis and evaluation of anti-enzymatic and antioxidant bioactivities. South African Journal of Botany. 2019 Jan 1; 120: 253-60.

[36] Jasicka-Misiak I, Poliwoda A, Petecka M, Buslovych O, Shlyapnikov VA, Wieczorek PP. Antioxidant phenolic compounds in Salvia officinalis L. and Salvia sclarea L. Ecological Chemistry and Engineering. 2018; 25 (1): 133.

[37] Hedhili L, Romdhane M, Abderrabba A, Planche H, Cherif I. Variability in essential oil composition of Tunisian Thymus capitatus (L.) Hoffmanns. et Link. Flavour and fragrance journal. 2002 Jan; 17 (1): 26-8.

[38] Amarti F, Satrani B, Aafi A, Ghanmi M, Farah A, Aberchane M, Ajjouri M, Antry S, Chaouch A. Chemical composition and antimicrobial activity of the essential oils of moroccan Thymus capitatus and Thymus bleicherianus. Phytothérapie. 2008; 6 (6): 342.

[39] Goudjil MB, Zighmi S, Hamada D, Mahcene Z, Bencheikh SE, Ladjel S. Biological activities of essential oils extracted from Thymus capitatus (Lamiaceae). South African Journal of Botany. 2020 Jan 1; 128: 274-82.

[40] Jabri-Karoui I, Bettaieb I, Msaada K, Hammami M, Marzouk B. Research on the phenolic compounds and antioxidant activities of Tunisian Thymus capitatus. Journal of Functional Foods. 2012 Jul 1; 4 (3): 661-9.

[41] Msaada K, Tammar S, Salem N, Bachrouch O, Sriti J, Hammami M, Selmi S, Azaiez S, Hadj-Brahim A, Al Sane K, Limam F. Chemical composition and antioxidant activities of Tunisian Thymus capitatus L. methanolic extract. International Journal of Food Properties. 2016 Jun 2; 19 (6): 1381-90.

[42] Saller, R. Peppermint (Mentha x piperita), medicinal plant of the year 2004, 11: 6–7.

[43] Toroglu S. In-vitro antimicrobial activity and synergistic/antagonistic effect of interactions between antibiotics and some spice essential oils. Journal of Environmental Biology. 2011 Jan 1; 32 (1): 23-9.

[44] Ahmad N, Fazal H, Ahmad I, Abbasi BH. Free radical scavenging (DPPH) potential in nine Mentha species. Toxicology and Industrial Health. 2012 Feb; 28 (1): 83-9.

[45] Shahrajabian MH, Sun W, Cheng Q. Chemical components and pharmacological benefits of Basil (Ocimum basilicum): a review. International Journal of Food Properties. 2020 Jan 1; 23 (1): 1961-70.

[46] Carović-Stanko K, Orlić S, Politeo O, Strikić F, Kolak I, Milos M, Satovic Z. Composition and antibacterial activities of essential oils of seven Ocimum taxa. Food Chemistry. 2010 Mar 1; 119 (1): 196-201.

[47] Trakoontivakorn G, Tangkanakul P, Nakahara K. Changes of antioxidant capacity and phenolics in Ocimum herbs after various cooking methods. Japan Agricultural Research Quarterly: JARQ. 2012; 46 (4): 347-53.

[48] Rao BR, Kothari SK, Rajput DK, Patel RP, Darokar MP. Chemical and biological diversity in fourteen selections of four Ocimum species. Natural product communications. 2011 Nov; 6 (11).

[49] Yin H, Fretté XC, Christensen LP, Grevsen K. Chitosan oligosaccharides promote the content of polyphenols in Greek oregano (Origanum vulgare ssp. hirtum). Journal of agricultural and food chemistry. 2012 Jan 11; 60 (1): 136-43.

[50] 51. Esen G, Azaz AD, Kurkcuoglu M, Baser KH, Tinmaz A. Essential oil and antimicrobial activity of wild and cultivated Origanum vulgare L. subsp. hirtum (Link) letswaart from the Marmara region, Turkey. Flavour and Fragrance Journal. 2007 Sep; 22 (5): 371-6.

[51] Bozin B, Mimica-Dukic N, Samojlik I, Jovin E. Antimicrobial and antioxidant properties of rosemary and sage (Rosmarinus officinalis L. and Salvia officinalis L., Lamiaceae) essential oils. Journal of agricultural and food chemistry. 2007 Sep 19; 55 (19): 7879-85.

[52] Rota C, Carraminana JJ, Burillo J, Herrera A. In vitro antimicrobial activity of essential oils from aromatic plants against selected foodborne pathogens. Journal of food protection. 2004 Jun; 67 (6): 1252-6.

[53] Yamamoto J, Yamada K, Naemura A, Yamashita T, Arai R. Testing various herbs for antithrombotic effect. Nutrition. 2005 May 1; 21 (5): 580-7.

[54] Haloui M, Louedec L, Michel JB, Lyoussi B. Experimental diuretic effects of Rosmarinus officinalis and Centaurium erythraea. Journal of ethnopharmacology. 2000 Aug 1; 71 (3): 465-72.

[55] Bakırel T, Bakırel U, Keleş OÜ, Ülgen SG, Yardibi H. In vivo assessment of antidiabetic and antioxidant activities of rosemary (Rosmarinus officinalis) in alloxan-diabetic rabbits. Journal of ethnopharmacology. 2008 Feb 28; 116 (1): 64-73.

[56] Fahim F, Esmat A, Fadel H, Hassan K. Allied studies on the effect of Rosmarinus officinalis L. on experimental hepatotoxicity and mutagenesis. International journal of food sciences and nutrition. 1999 Jan 1; 50 (6): 413-27.

[57] Perez-Fons L, Garzon MT, Micol V. Relationship between the antioxidant capacity and effect of rosemary (Rosmarinus officinalis L.) polyphenols on membrane phospholipid order. Journal of agricultural and food chemistry. 2010 Jan 13; 58 (1): 161-71.

[58] Yosr Z, Hnia C, Rim T, Mohamed B. Changes in essential oil composition and phenolic fraction in Rosmarinus officinalis L. var. typicus Batt. organs during growth and incidence on the antioxidant activity. Industrial Crops and Products. 2013 May 1; 43: 412-9.

[59] Lagouri V, Alexandri G. Antioxidant properties of greek O. dictamnus and R. officinalis methanol and aqueous extracts—HPLC determination of phenolic acids. International Journal of Food Properties. 2013 Jan 1; 16 (3): 549-62.

[60] Khalil R, Li ZG. Antimicrobial activity of essential oil of Salvia officinalis L. collected in Syria. African Journal of Biotechnology. 2011; 10 (42): 8397-402.

[61] Bouajaj S, Benyamna A, Bouamama H, Romane A, Falconieri D, Piras A, Marongiu B. Antibacterial, allelopathic and antioxidant activities of essential oil of Salvia officinalis L. growing wild in the Atlas Mountains of Morocco. Natural Product Research. 2013 Sep 1; 27 (18): 1673-6.

[62] Pereira OR, Catarino MD, Afonso AF, Silva A, Cardoso SM. Salvia elegans, Salvia greggii and Salvia officinalis decoctions: Antioxidant activities and inhibition of carbohydrate and lipid metabolic enzymes. Molecules. 2018 Dec; 23 (12): 3169.

[63] Jug Dujaković M, Ristić M, Pljevljakušić D, Dajić Stevanović Z, Liber Z, Hančević K, Radić T, Šatović Z. High diversity of indigenous populations of dalmatian sage (Salvia officinalis L.) in essential oil composition. Chemistry & biodiversity. 2012 Oct; 9 (10): 2309-23.

[64] Bouyahya A, Chamkhi I, Guaouguaou FE, Benali T, Balahbib A, El Omari N, Taha D, El-Shazly M, El Menyiy N. Ethnomedicinal use, phytochemistry, pharmacology, and food benefits of Thymus capitatus. Journal of Ethnopharmacology. 2020 Sep 15; 259: 112925.

[65] Džamić AM, Nikolić BJ, Giweli AA, Mitić Ćulafić DS, Soković MD, Ristić MS, Knežević Vukčević JB, Marin PD. Libyan Thymus capitatus essential oil: antioxidant, antimicrobial, cytotoxic and colon pathogen adhesion inhibition properties. Journal of applied microbiology. 2015 Aug; 119 (2): 389-99.

[66] Gonçalves JC, de Meneses DA, de Vasconcelos AP, Piauilino CA, Almeida FR, Napoli EM, Ruberto G, de Araújo DA. Essential oil composition and antinociceptive activity of Thymus capitatus. Pharmaceutical biology. 2017 Jan 1; 55 (1): 782-6.

[67] Iauk L, Acquaviva R, Mastrojeni S, Amodeo A, Pugliese M, Ragusa M, Loizzo MR, Menichini F, Tundis R. Antibacterial, antioxidant and hypoglycaemic effects of Thymus capitatus (L.) Hoffmanns. Et Link leaves' fractions. Journal of Enzyme Inhibition and Medicinal Chemistry. 2015 May 4; 30 (3): 360-5.

[68] Proestos C, Chorianopoulos N, Nychas GJ, Komaitis M. RP-HPLC analysis of the phenolic compounds of plant extracts. Investigation of their antioxidant capacity and antimicrobial activity. Journal of agricultural and food chemistry. 2005 Feb 23; 53 (4): 1190-5.

[69] Nouraei S, Rahimmalek M, Saeidi G. Variation in polyphenolic composition, antioxidants and physiological characteristics of globe artichoke (Cynara cardunculus var. scolymus Hayek L.) as affected by drought stress. Scientia Horticulturae. 2018 Mar 15; 233: 378-85.

[70] Gul MZ, Chandrasekaran S, Bhat MY, Maurya R, Qureshi IA, Ghazi IA. Antioxidant and enzyme inhibitory activities of Cissampelos pareira L. leaf extracts. Ann. Phytomed. 2016 Jan 1; 5 (1): 91-8.

[71] Su C, Gius JP, Van Steenberg J, Haskins AH, Heishima K, Omata C, Iwayama M, Murakami M, Mori T, Maruo K, Kato TA. Hypersensitivity of BRCA2 deficient cells to rosemary extract explained by weak PARP inhibitory activity. Scientific reports. 2017 Dec 1; 7 (1): 1-8.

[72] Ullah S, Rahman KU, Rauf A, Hussain A, Ullah A, Fawzy M. Phenolic acids, flavonoids and antiradical activity of Ocimum sanctum and Ocimum basilicium leaves extract. ZEITSCHRIFT FUR ARZNEI-& GEWURZPFLANZEN. 2020 Jan 1; 25 (2): 60-2.

[73] Gómez-Estaca J, Bravo L, Gómez-Guillén MC, Alemán A, Montero P. Antioxidant properties of tuna-skin and bovine-hide gelatin films induced by the addition of oregano and rosemary extracts. Food Chemistry. 2009 Jan 1; 112 (1): 18-25.

[74] Khiya Z, Oualcadi Y, Gamar A, Berrekhis F, Zair T, Hilali FE. Correlation of total polyphenolic content with antioxidant activity of hydromethanolic extract and their fractions of the Salvia officinalis leaves from different regions of Morocco. Journal of Chemistry. 2021 Feb 8; 2021.

[75] Bianchin M, Pereira D, Almeida JD, Moura CD, Pinheiro RS, Heldt LF, Haminiuk CW, Carpes ST. Antioxidant Properties of Lyophilized Rosemary and Sage Extracts and its Effect to Prevent Lipid Oxidation in Poultry Pâtê. Molecules. 2020 Jan; 25 (21): 5160.

[76] Park JB. Identification and quantification of a major anti-oxidant and anti-inflammatory phenolic compound found in basil, lemon thyme, mint, oregano, rosemary, sage, and thyme. International journal of food sciences and nutrition. 2011 Sep 1; 62 (6): 577-84.

[77] Roby MH, Sarhan MA, Selim KA, Khalel KI. Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (Thymus vulgaris L.), sage (Salvia officinalis L.), and marjoram (Origanum majorana L.) extracts. Industrial Crops and Products. 2013 May 1; 43: 827-31.

[78] Oniga I, Puşcaş C, Silaghi-Dumitrescu R, Olah NK, Sevastre B, Marica R, Marcus I, Sevastre-Berghian AC, Benedec D, Pop CE, Hanganu D. Origanum vulgare ssp. vulgare: Chemical composition and biological studies. Molecules. 2018 Aug; 23 (8): 2077.

[79] Teofilović B, Grujić-Letić N, Karadžić M, Kovačević S, Podunavac-Kuzmanović S, Gligorić E, Gadžurić S. Analysis of functional ingredients and composition of Ocimum basilicum. South African Journal of Botany. 2021 Sep 1; 141: 227-34.

[80] Hawrył MA. HPLC-diode array detector fingerprints of various Mentha species. Journal of AOAC International. 2014 Sep 1; 97 (5): 1268-73.

[81] Habib, H. I. I., Omar, S. K., & Mohamed, H. S. Estimation of rutin and ascorbic acid in some libyan herbal plants by RP-HPLC. *Med Aromat Plants*, 2016, *5* (4), 255-259.

[82] Babili FE, Bouajila J, Souchard JP, Bertrand C, Bellvert F, Fouraste I, Moulis C, Valentin A. Oregano: chemical analysis and evaluation of its antimalarial, antioxidant, and cytotoxic activities. Journal of food science. 2011 Apr; 76 (3): C512-8.

[83] Armatu A, Colceru-Mihul S, Bubueanu C, Draghici E, Pirvu L. Evaluation of antioxidant and free scavenging potential of some Lamiaceae species growing in Romania. Romanian Biotechnological Letters. 2010 May 1; 15 (3): 5274-80.

[84] Kumaran A, Karunakaran RJ. In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. LWT-Food Science and Technology. 2007 Mar 1; 40 (2): 344-52.