

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

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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. ($0.59 \text{ mg/mL} \pm 0.01$), *Salvia officinalis* L. ($0.60 \text{ mg/mL} \pm 0.01$), *Rosmarinus officinalis* L ($0.65 \text{ mg/mL} \pm 0.07$), and *Ocimum basilicum* ($0.99 \text{ mg/mL} \pm 0.02$). 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 flavonoids
<i>M. piperita</i>	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].
<i>O. bacsilicum</i>	Chavicol methyl ether (estragol), linalool, eugenol, 1, 8- cineole, methyl cinnamate and linalool [19, 20].	Caftaric, caffeic, gentisic, <i>p</i> -coumaric, ferulic and chlorogenic acids), (rutin, quercitin and isoquercitrin), and free flavonoid aglycons (luteolin, quercetin) [21].
<i>O. vulgare L</i>	Carvacrol, thymol, linalyl acetate, (<i>Z</i>)- α -bisabolene, (<i>E</i>)- 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].
<i>R. officinalis</i>	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].
<i>S. officinalis</i>	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, epicatechin, rosmarinic acid, quercetin, rutin and luteolin-7-glucoside [34], <i>p</i> -hydroxybenzoic acid, 3-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, syringic acid, caffeic acid, <i>p</i> -coumaric acid, rosmarinic acid, vanillic acid, ferulic acid), and flavonoids (kaempferol, chrysin, galangin, myricetin, luteolin and pinocembrin) [36].
<i>T. capitatus</i>	Thymol, Carvacrol, γ -terpinene, <i>p</i> -cymene, linalool and β -caryophyllene [31, 37-39].	Tannic acid, gallic acid, chlorogenic hemihydrate, caffeic acid, syringic acid as well as ferulic acid, <i>p</i> -coumaric acid, and <i>trans</i> -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
<i>M. piperita</i>	Antimicrobial, sedative, analgesic, carminative [42].	Antiallergic [15], antimicrobial [43], antioxidant [44].
<i>O. bacsilicum</i>	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].
<i>O. vulgare L</i>	Treatment of cold, cough, and digestive disorders [49].	Antimicrobial and antioxidant activities [25, 50], anti-ulcer, anti-inflammatory, antidiabetic, antiviral, cytotoxic and antitumour [26].
<i>R. officinalis</i>	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].
<i>S. officinalis</i>	Treatment of seizure, ulcers, gout, inflammation, diabetes, dizziness, tremor, paralysis and diarrhea [34].	Antimicrobial [34, 51, 52, 61], antioxidant [35, 61, 62], antidiabetic, anti-obesity, gastroprotective, antispasmodic, anti-inflammatory, virucidal, fungicidal, and bactericidal [63], antimutagenic, antidementia, hypoglycemic, and hypolipidemic effects [34].
<i>T. capitatus</i>	Anthelmintic, carminative, antispasmodic, emmenagogue, rubefactant, 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, rosemary, 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 methanolic 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 μ L 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 μ m, 250 mm \times 4.6 mm \varnothing). 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 μ L 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:

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

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 (μ g 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.

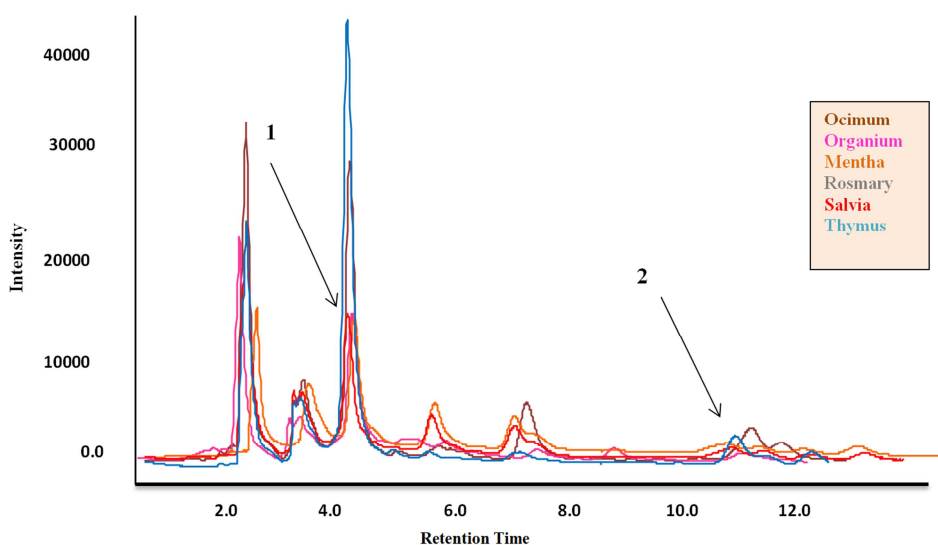


Figure 1. RP-HPLC chromatogram of the methanolic extracts of the plants for investigation of Gallic acid and Rutin.

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

Peak	R _t	Area under the peak (%) in the different plants					
		<i>Ocimum</i>	<i>Organium</i>	<i>Mentha</i>	<i>Rosmary</i>	<i>Salvia</i>	<i>Thymus</i>
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 IC₅₀ ± SD.

plant	DPPH IC ₅₀ (mg/mL)
<i>Ocimum basilicum</i>	0.99 ± 0.02
<i>Organium vulgare</i>	0.34 ± 0.03
<i>Mentha piperta</i>	0.59 ± 0.01
<i>Rosmarinus officinalis</i>	0.65 ± 0.07
<i>Salvia officinalis</i>	0.60 ± 0.01
<i>Thymus capitatus</i>	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 rosmariny 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 rosmariny, 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 (Lamiaceae) 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.

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