OPEN ACCESS
Global Journal of Research in Medical Sciences
ISSN: 2583-3960 (Online)
Volume 05 | Issue 01 | Jan.-Feb. | 2025
Journal homepage: https://gjrpublication.com/gjrms/
Research Article

Phytochemical Composition, Antioxidant Activity and HPLC of *Matricaria recutita L.* and leaves of *Ocimumsanctum L*

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DOI: 10.5281/zenodo.14901383 **Submission Date: 28 Dec. 2024 | Published Date: 20 Feb. 2025**

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Abstract
The present study was performed to investigate the variation of phytochemical composition, antioxidant activity and High-Performance Liquid Chromatography (HPLC) of Matricaria recutita L. and the leaves of Ocimumsanctum L, in addition to diet, they are used as medicinal plant. The phytochemical screening was assessed by using different extracts: aqueous and acetone. The total phenolic content was determined spectrophotometrically by Folin-Ciocalteu's method and the total flavonoid content were also determined by using the aluminum chloride complex formation assay. In addition, as compared to the well-known antioxidant ascorbic acid, the extracts of Matricaria recutita L had greater antioxidant activity than extracts of leaves of Ocimumsanctum in radical scavenging activity by DPPH, where the DPPH radical scavenging activity ranged from 92 % at 500 g/ml of the aqueous extracted from Chamomile, 67 % of the acetone extract at 500 g/ml, 88 % of the aqueous extract from leaves of Ocimum at 500 g/ml, and 62 % of the acetone extract at 500 g/ml.

Keywords: *Phytochemistry Chamomile, leaves Ocimum, folin-ciocalteu and DPPH.*

Introduction
Cells generate free radicals on a regular basis as part of their usual functions. Around 95 percent of oxygen absorbed by tissues is used in metabolic processes, but only about 5% of that oxygen is converted into reactive species [1]. ROS/RNS are considered to play a dual role in biological processes, as they can either damage or benefit living organisms. ROS's beneficial effects include physiological functions in cellular responses, such as protection against infectious agents and the operation of many cellular signaling systems [2]. Reactive oxygen species (ROS) and oxidative stress are also important factors in the pathology and development of a variety of human diseases. As a result, huge efforts have been made to scientifically examine possible endogenous and exogenous antioxidants [3]. Antioxidants are compounds that prevent the initiation or propagation of oxidative chain reactions, thus delaying or inhibiting the oxidation of lipids or other molecules. They serve as reducing agents, scavengers of free radicals, and quenchers of singlet oxygen in one or more ways [4]. *Matricaria chamomilla* L. is a well-known folk medicine plant that is grown all over the world. The pharmaceutical, cosmetic, and food industries all use *chamomile* essential oil. *Chamomile's* pharmacological effect is primarily linked to its essential oil, which has spasmolytic, antimicrobial, and disinfectant properties. *Chamomile* essential oil contains biologically active substances [5]. The extract of *Matricaria chamomilla* L. is high in flavonoids, terpenes, and polysaccharides, among other things, which may contribute to the formulation's bioactive properties, such as anti-inflammatory and emollient effects. [6,7,8]. Antimicrobial properties have been identified for α-bisabolol, an isolated compound from chamomile extract [9]. anti-inflammatory, anti-nociceptive [10], and antimalarial [11] things to do by Apigenin, another isolated compound from chamomile extract, which has been reported to protect skin keratinocytes from UVB-induced damage, DNA damage and reactive oxygen species [12]. The extract of *Ocimumsanctum* L. has a high potential to scavenge highly reactive free radical [13]. The antioxidant activity of *Ocimum* extract of fresh leaves and stems included phenolic compounds such as irsilineol, cirsimaritin, isothymusin, apigenin, and rosmarinic acid, as well as significant amounts of eugenol (a major component of the volatile oil) [14]. The antioxidant ability of essential oils obtained by steam hydro distillation from *Ocimum sanctum* L. was assessed using DPPH (1,1-Diphenyl-2-picrylhydrazyl) assays, and it was discovered that O. *sanctum* L. had a high antioxidant capacity in the

hypoxanthine/ xanthine oxidase assay [15]. Another research discovered that an aqueous extract of *Ocimum* sanctum L. substantially increased anti-oxidant activity. [16].

MATERIALS AND METHODS

1. Materials:

1.1 Plant material:

The *Chamomile* (*Matricaria recutita L.*) and the leaves of *Ocimumsanctum* L., were collected from Al-Jabal Al Akhdar area in Libya 2016.

1.2. Chemicals:

The 1,1-diphenylpicrylhydrazyl (DPPH) was given by Sigma and Merck company.
The biochemistry laboratory of the chemistry department-Benghazi provided ascorbic acid, Folin-Ciocalteu reagent, ferric chloride, potassium ferricyanide, monobasic dihydrogen phosphate, dibasic monohydrogen phosphate, trichloro acetic acid, sodium carbonate, petroleum ether, anhydrous sodium sulfate, and pyrogallol.

2. Methods:

2.1. Sample preparation:

2.1.1. Extraction of samples:

The dry powdered *Chamomile* and *Ocimum* leaves were extracted with aqueous extract: powdered plants (50g) were extracted with 250 ml distilled water for 12 hours using a Soxhelt extractor (size 29-24). The liquid was then evaporated to dryness using a Rotatory evaporator at 70-80°C (RE2000). Acetone extract: For 12 hours, the powdered plants (40g) were extracted with acetone. The extracts were dried in a rotatory evaporator at 40 ^{O}C.

2.2. Extracts analysis:

The samples extracted from *Chamomile* and leaves of *Ocimum* were subjected to:

2.2.1. HPLC chromatography/ Mass spectra.

Thermo Scientific, Trace HPLC Ultra & ISQ Single Quadruple MS, DB-5 bonded-phase fused-silica capillary column was used in for HPLC/MS analysis of the samples (Acetone extracted) in Central laboratory cairo University.

2.2.2. Antioxidant activities assays and quantitative analysis:

All of these experimental have been conducted in (Judicial experience and research center, Benghazi/Libya).

2.2.2.1. Total phenolic content (TPC):

Total concentration of phenolic compound in the extracts obtained from of *Chamomile* and leaves of *Ocimum* were estimated using the colorimetric method based on Folin-Ciocalteu reagent [17]. 0.05 ml of the aqueous extracted from *chamomilla* and *Ocimum* at different concentrations "100, 200, 300, 400, 500 µg/ml" were mixed separately with 0.05ml of Folin-Ciocalteu reagent, then 0.5ml of 15% sodium carbonate solution was added to the mixture and then the volum was adjusted to 1ml with 0.4ml of distilled water. The reaction was allowed to stand for 10 min, after which the absorbance were recorded at 725nm by UV-visible spectrophotometer. Quantification was done with respect to standard calibration curve of Pyrogallol, the results were expressed as pyrogallol "µg/ml". Estimation of the phenolic compounds was carried out in triplicate. The results were mean values ± standard deviations.

2.2.2.2. Total flavonoids content (TFC):

Aluminum chloride colorimetric method was used for total flavonoid determination [18]. 2ml of different concentration "100, 200, 300, 400, 500 µg/ml "of aqueous extracted from *chamomilla* and *Ocimum* mixed with 0.1ml of 10% aluminum chloride, 0.1ml of 1M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30min; the absorbance of the reaction mixture was measured at 415nm with a UV-visible spectrophotometer. The calibration curve was obtained by preparing different quercetin solutions in methanol at concentrations "100 to 500 µg/ml".

2.2.2.3. Reducing power assay (RPA):

The reducing power was determined according to the Naznin et al. method [19]. 2ml of aqueous extracted from *chamomilla* and *Ocimum* with different concentration "100, 200, 300, 400, 500 µg/ml" was mixed with 2.5ml phosphate buffer (0.2M, pH 6.6) and 2.5 ml potassium ferricyanide, then the mixture was incubated in water bath at 50^{0}C for 20 minutes. 2.5ml of trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. Finally, 2.5ml of the supernatant was mixed with 2.5ml of distilled water and 1ml $FeCl_3$, substances, which have a reduction potential to react with potassium ferricyanide (Fe^{3+}) to form potassium ferricyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700nm by UV-Visible

spectrophotometer. Quantification was done with respect to standard calibration curve of ascorbic acid the results were expressed as ascorbic acid "µg/ml".

Potassium ferricyanide + ferric chloride $\xrightarrow{\text{antioxidant}}$ potassium ferricyanide + ferrous chloride.

2.2.2.4. DPPH free radical scavenging activity (RSA):

The antioxidant activity of the all extracts were measured in terms of hydrogen donating or radical-scavenging ability using the stable DPPH˙ method as modified by Park et al. [20]. The reaction mixture containing 2ml of the oil at different concentration "100, 200, 300, 400, 500 µg/ml" and 2ml of DPPH˙ (0.2mM) was vigorously shaken and incubated in darkness at room temperature for 30 minutes. When the DPPH˙ reacted with an antioxidant compound in the oil that can donate hydrogen, it was reduced and resulting a decrease in absorbance at 517nm using UV-visible spectrophotometer, and the mean values were obtained from triplicate experiments. The percentage of the remaining DPPH˙ was plotted against the sample concentration. A lower value indicates greater antioxidant activity. Radical scavenging activity was expressed as percent of inhibition and was calculated using the following formula: -

%DPPH "RSA" = [Abs. of Control – Abs. of Sample / Abs. of Control] x 100

RESULTS

1.Antioxidant evaluation of *Chamomile* and leaves of *Ocimumsanctum* L. extracts (aqueous and acetone).

The antioxidant activities of all extracts of *Matricaria chamomilla* L. and *Ocimumsanctum* L. grows are evaluated by:

1.1.Total phenolic content (TPC):

Table (1) show the total phenolic content that found in all extracted from *Matricaria chamomilla* L. and *Ocimumsanctum* L., were the aqueous extracted from *Chamomile* and *Ocimum* contain high total phenolic content, the results expressed according to pyrogallol as phenolic compound.

1.2. Total flavonoids content (TFC):

The results obtained in this study as shown in Table (2) indicate that the aqueous solution and acetone extracted from *Chamomile* and *Ocimum*contain have high amount of flavonoids compounds as compared with the quercetin, which used as standard.

1.3. Reducing power assay (RPA):

As shown in Table (3) the reducing power assay of aqueous solution and acetone extracted from *Chamomile* and *Ocimum*exhibit have higher reducing activity than the ascorbic acid.

1.4. The DPPH˙ radical scavenging activity:

The result of the DPPH˙ radical scavenging activity of aqueous solution and acetone extracted from *Chamomile* and *Ocimum*are shown in Table (4). These results compared with the well - known antioxidant ascorbic acid, where the percent of the inhibition is 92% at 500 µg/ml of the aqueous extracted from *Chamomile*, 67% at 500 µg/ml of the acetone extract, while 88% at 500 µg/ml of the aqueous extracted from the leaves of *Ocimum*, 62% of the acetone extract.

Table 1: Total phenolic content (Mean ±Standard Deviation, N=3) of aqueous solution and acetone extracted from *Chamomile* and *Ocimum* and pyrogallol as standard

Concentration (µg/ml)	Pyrogallol	Aqueous Extract		Acetone Extract	
		Chamomile	Leaves of *Ocimum*	*Chamomile*	Leaves of *Ocimum*
100	**0.438 ± 0.024**	**0.352 ± 0.046**	**0.302 ±0.026**	**0.293 ± 0.023**	**0.253 ± 0.073**
200	**0.725 ± 0.037**	**0.612± 0.062**	**0.572± 0.032**	**0.532 ± 0.032**	**0.502 ± 0.022**
300	**1.070 ± 0.021**	**0.843± 0.052**	**0.821± 0.051**	**0.812 ± 0.031**	**0.772 ± 0.051**
400	**1.307 ± 0.019**	**1.19± 0.034**	**1.15 ± 0.065**	**1.12 ± 0.051**	**1.02 ± 0.091**
500	**1.564 ± 0.027**	**1.35± 0.066**	**1.35 ± 0.029**	**1.28 ± 0.047**	**1.20 ± 0.027**

Table 2: Total Flavonoid content of aqueous solution and acetone extracted from *Chamomile*, Ocimum, and Quercetin as standard

Concentration (µg/ml)	Quercetin	Aqueous Extract		Acetone Extract	
		Chamomile	Leaves of *Ocimum*	*Chamomile*	Leaves of *Ocimum*
100	0.307 ± 0.074	0.273 ± 0.022	0.265 ± 0.056	0.235 ± 0.083	0.226 ± 0.027
200	0.612 ± 0.027	0.482 ± 0.074	0.356 ± 0.032	0.344 ± 0.053	0.308 ± 0.031
300	0.954 ± 0.077	0.712 ± 0.083	0.678 ± 0.072	0.447 ± 0.023	0.438 ± 0.097
400	1.203 ± 0.082	1.021 ± 0.053	1.097 ± 0.035	0.788 ± 0.095	0.756 ± 0.053
500	1.511 ± 0.033	1.301 ± 0.026	1.227 ± 0.021	0.972 ± 0.056	0.974± 0.092

Table 3: Reducing power assay of aqueous solution and acetone extracted from *Chamomile*, Ocimum, and Vitamin C as standard

Concentration (µg/ml)	Vitamin C	Mean ± Standard Deviation of Aqueous Extract		Mean ± Standard Deviation of Acetone Extract	
		Chamomile	Leaves of *Ocimum*	*Chamomile*	Leaves of *Ocimum*
100	0.468 ± 0.044	0.398 ± 0.033	0.381 ± 0.067	0.373 ± 0.044	0.312 ± 0.033
200	0.712 ± 0.067	0.605 ± 0.062	0.587 ± 0.071	0.618 ± 0.029	0.593 ± 0.092
300	0.972 ± 0.027	0.801 ± 0.043	0.792 ± 0.077	0.735 ± 0.059	0.771 ± 0.042
400	1.22 ± 0.062	0.971± 0.066	0.853 ± 0.083	0.948 ± 0.028	0.983 ± 0.055
500	1.51 ± 0.073	1.32± 0.045	1.22 ± 0.062	1.21± 0.055	1.26 ± 0.061

Table 4: Percent of DPPH˙radical inhibition by of aqueous solution and acetone extracted from *Chamomile*, Ocimum, and Vitamin C as standard

Concentration (µg/ml)	Vitamin C	Aqueous Extract		Acetone Extract	
		Chamomile	Leaves of *Ocimum*	*Chamomile*	Leaves of *Ocimum*
100	32 %	32.5 %	29.2 %	22.4 %	17 %
200	46 %	49.4 %	46.4 %	32.3 %	23.5 %
300	61 %	68.4 %	59 %	43 %	31 %
400	75 %	85 %	77.7 %	56 %	41.5 %
500	87 %	92 %	88 %	67 %	62.4 %

The HPLC chromatography/ Mass spectra of the acetone extracted from *Chamomile* and *Ocimum*:

Table (5) represents the chemical composition of the acetone extracted from *Chamomile*. As can be seen from this table, the compounds representing about 99.61% of the acetone extracted from *Chamomile*. The major components are as follows: Bisabolol oxide A (22.78%), Cis-Spiroether (En-yn-dicycloether (25%), Sesquiterpenes (17%), Apigenin (16%).

Table (6) shows the results obtained from the **HPLC chromatography/ Mass spectra** for the acetone extracted from *Ocimum* where the results showed that the acetone extracted from *Ocimum* contains 10 compounds, one of the most important of these compounds are eugenol (47.55), caryophyllene oxide (24.56), 1,2,4-triethenyl Cyclohexane (15.22) and 2-methyhlpentanedinitrile (5.08).

Table (5): The chemical constituent of the acetone extracted from *Chamomile* by HPLC chromatography/ Mass spectra

No.	RT	Name of the compound	Peak Area %
1	**14.57**	Bisabolol oxide B	0.3
2	**14.89**	Alpha-bisabolol	4.1
3	**15.46**	Herniarin	0.82
4	**15.65**	Bisabolol oxide A	22.78

5	**16.80**	Umbelliferone	3.09
6	**17.02**	Cis-Spiroether (En-yn-dicycloether)	25
7	**17.13**	Trans-Spiroether (En-yn-dicycloether)	5.46
8	**18.52**	(E)-β-farnesene	1.22
9	**19.74**	Achillin	1.52
10	**21.67**	Matricarin	1.02
11	**26.10**	Chamazulene	1.3
12	**34.72**	Sesquiterpenes	17
14	**38.12**	Apigenin	16

RT = retention time; Conc. (%) based on peak area integration.

Table (7): The chemical constituent of acetone extracted from Ocimum by HPLC chromatography/ Mass spectra

No.	RT	Name of the compound	Peak Area %
1	6.10	Eugenol	47.55
2	6.33	Cyclohexane, 1,2,4-triethenyl	15.22
3	7.09	Caryophyllene	24.56
4	7.59	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	1.00
5	9.11	Cyclopentane, cyclopropylidene-	0.97
6	14.95	Z, Z-4,16-Octadecadien-1-ol acetate	0.66
7	20.71	Benzene methanamine, N, N-a,4-tetramethyl-	1.86
8	20.85	3,8,8-trimethoxy-3-piperidyl-2,2-binaphthalene1,1,4,4-tetrone	1.05
9	24.21	Octadecane, 1,1-dimethoxy-	2.05
10	24.78	Pentanedinitrile, 2-methyl-	5.08

RT = retention time; Conc. (%) based on peak area integration.

Discussion

Antioxidant phytochemicals are critical for human health because of their scavenging ability. In vitro antioxidant screening revealed that aqueous extracts of *chamomilla* and *Ocimum* contain significant amounts of polyphenolic and flavonoids compounds, which are responsible for the antioxidant properties. In addition, because of their reducing ability and DPPH free radical scavenging activity, they have a higher reductive potential, which is a good indicator of antioxidant activity. The terpenoid group of antioxidants, which includes chamazulene and acetylene derivatives, are the most important antioxidant components derived from *chamomile* flowers. Several phenolic compounds, especially flavonoids such as apigenin, quercetin, and patuletin, as well as various glucosides, are other major constituents of the plants [22]. These compounds reduce inflammation by preventing cell mutation and fighting free radical damage. The antioxidants in *chamomile* are linked to improved immune function, lower rates of mood disorders, decrease pain and swelling, and healthier skin. [23]. The phenolic and flavonoids compounds are classes of secondary metabolites with a wide range of biological properties, including antioxidant, anti-atherosclerosis, cardiovascular defense, and endothelial function improvement. It has been documented that the phenolic compounds' antioxidant activity is primarily due to their redox properties, which enable them to act as reducing agents, Adsorbing and neutralizing reactive free radicals, as well as chelating ferric ions, which catalyze lipid peroxidation, hydrogen donors are regarded as a potential therapeutic agent for free radical-linked pathologies [24]. Sesquiterpenes, apigenin, bisabolol oxide A from chamomilla extract and eugenol, Caryophyllene, Cyclohexane, 1,2,4-triethenyl from *Ocimum* extract are the most important components, according to the HPLC-MS results. According to researchers, the high concentration of apigenin and eugenol in the extract makes it potentially useful in medicines because they have antibacterial, antifungal, anti-inflammatory, and antioxidant properties [25, 26]. *Chamomile* is known as "the plant doctor" because it is thought to aid in the growth and health of a variety of other plants, especially those that produce essential oils. [27]. -pinene, -pinene, camphene, sabinene, myrcene, 1,8-cineole, y-terpinene, caryophyllene, propyl angelate, butyl angelate, chamazulene, a-bisabolol, bisabolol oxide A, bisabolol oxide B, and bisabolone oxide are the major chemical components of *chamomile* extraction oils [28]. Cardiovascular, cancer, hyperlipidemia, diabetes, alzheimer's, and inflammatory diseases can grow in the body when there aren't enough antioxidants to quench the excess reactive free radicals [29]. A potential mechanism for *Chamomilla* and *Ocimum* components acting as free radical scavengers intercepting certain free radicals may be a mechanism for defense by aqueous solution derived from chamomilla and *Ocimum* against liver damage [30, 24]. The total phenolic content was measured using the Folin-Ciocalteu reagent, which creates a blue color when Molybdenum "Mo" is reduced to Mo(V) by accepting an electron from the reducer (i.e. antioxidant), and the lower absorbance at 517 nm suggests greater radical scavenging activity. Finally, its antioxidant activity will provide us with useful details regarding its radical scavenging activity. [31].

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CITATION
Maraia, F. E., Elwerfally, O. B., Salem G. El T., Naema M El A., & Layas, Y. F. (2025). Phytochemical Composition, Antioxidant Activity and HPLC of Matricaria recutita L. and leaves of Ocimumsanctum L. In Global Journal of Research in Medical Sciences (Vol. 5, Number 1, pp. 96–102).
https://doi.org/10.5281/zenodo.14901383