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Investigations of the Palestinian medicinal plant basil (*Ocimum basilicum*): antioxidant, antimicrobial activities, and their phase behavior

Abstract

Objective: The aim of this work was to establish pseudoternary phase diagrams for Palestinian basil extracts (seeds and leaves), formulate a microemulsion, and study the antioxidant and antimicrobial effects of this medicinal plant.

Methods: Pseudoternary phase diagrams were prepared by utilizing the water titration method, by using basil extracts (seeds and leaves) as an oil phase and water phase. Basil was analyzed for its phytochemical constituents after it was extracted using Soxhlet extraction device. The crude extracts of basil (seeds and leaves) were analyzed for their total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (AA) using standard methods (Folin-Ciocalteau assay, aluminum chloride assay, ferric reducing/antioxidant power (FRAP) and spectrophotometric method), respectively. *In-vitro* susceptibility of basil extracts was determined by antibacterial activity using agar disc diffusion method, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) against five clinical pathogens. The potential *in-vitro* antimalarial activity of various *Ocimum* extracts was determined in different solvents using semiquantitative assay method.

Results: The results showed that both the seeds and leaves of the ethanol extract had antibacterial activity on most bacterial strains, with respective zones of inhibition of 6-25 mm and MIC values of (62.5 - > 500µg/ml). The seeds extract of the plant has 74±1.4 mg/g AA, while the leaves extract has a value of 40.4±0.8 mg/g. The TPC of basil seeds was 58.2±0.9 mg/g and that of basil leaves was 51±2.4 mg/g. In addition, the TFC in the leaves was 9.0±1.5 mg/g, while in the seeds it was 34.2±3.6 mg/g. At room temperature, the pseudoternary phase diagram was determined. Combining basil leaves and seeds extract with Tween 20 as a surfactant resulted in an effective basil microemulsion. RP-HPLC Chromatography analysis showed the presence of flavonoid (quercetin) in 6.6 min. It was found that β-hematin was not prevented in any of these solvents. This result, shown for the first time in this study, rules out a mechanism that has been proposed to explain the antimalarial activity of the plant, namely inhibition of beta-hematin formation.

Conclusion: Based on these results, it is concluded that basil is a natural source of potent antioxidants, antibacterial and antimalarial activities that can prevent many diseases and could potentially be used in cosmetics and pharmaceutical products.

Keywords: basil, phase behavior, microemulsion, antimalarial activity

Volume 10 Issue 3 - 2022

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Received: May 07, 2022 | **Published:** June 21, 2022

Introduction

Basil is a plant of the *Lamiaceae* family, belonging to the *Ocimum basilicum* L. family. This plant is a fragrant herb that reaches a height of 20-80cm.[1] In addition, the genus *Ocimum* includes 50-150 species of herbs and shrubs.[2,3] Due to the excellent climatic conditions in India and China, it is considered the original native basil, although today it is grown commercially in many tropical and temperate countries in Asia, Africa, and Central and South America.[4]

Several studies have addressed the phytochemical constituents of sweet basil essential oil fresh leaves and seeds. More than 200 chemical compounds have been identified, revealing a wide variety of constituents of the oil from different parts of the world, including monoterpenes, limonene, myrcene, terpinols, flavonoids, phenolic acids, vitamins, and steroids.[5]

Extraction of plant samples whether for leaves or seeds has been developed for decades using a variety of techniques. One of them is the conventional Soxhlet extraction. This technique is currently one of the most widely used extraction techniques for plants.[6] Not only in chemical analysis, but also in many other fields.[7] Pseudo-ternary phase diagrams are made with fixed weight ratios of co-surfactants and surfactants. Ternary phase diagrams are constructed to define the range of microemulsion.[8] Microemulsion, first introduced by Hoar and Schulman in 1943, is one of the practical dosage forms. A microemulsion is a transparent dispersion system consisting of an aqueous phase, oil, a surfactant, and a co-surfactant. It forms a single liquid solution, an optically isotropic liquid with droplet diameters typically ranging from 10 to 100 nm, and thermodynamically stable systems.[9] In a microemulsion, there is a specific boundary surfactant between the oil and aqueous phases, which gives the microemulsion a particular microstructure.[10]

Basil is generally used to treat a variety of ailments. Antioxidant, antibacterial, antimalarial, and hypercholesterolemic properties have been documented.[11] Essential plant oils are being explored as a promising alternative to currently used antimicrobial agents. This activity is attributed to their ability to synthesize aromatic substances, most of which are phenols or oxygen-substituted derivatives.[12] Monoterpenes, sesquiterpenes, and their oxygenated derivatives form

the essential oils, which are a complex collection of substances.[13] Many species of *Ocimum* (including *O. basilicum*) essential oils have antimicrobial activity against multidrug-resistant clinical strains of many pathogenic microorganisms and possess a broad spectrum of *in-vitro* antimicrobial activities. They have also long been used as flavoring agents or preservatives in foods, beverages, and confectionery.[14]

Ocimum basilicum also possesses significant antioxidant properties,[15] which have a variety of therapeutic effects on the body, such as preventing cancer and reducing the incidence of diseases with the highest death rates worldwide.[16] Malaria is caused by parasites such as *Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale,* and *Plasmodium vivax. P. falciparum* is a Plasmodium protozoan that is transmitted to humans through the bite of an infected female Anopheles mosquito, which then introduces the parasite into the bloodstream of the human host.[17]

The parasites developed resistance to the most used antimalarial drugs, while the vectors developed resistance to pesticides. Antifolates and artemisinin have drawn attention to the urgent need for new antimalarial drugs to control this deadly disease.[18] Plant extracts are still commonly used in the treatment of malaria and other diseases. For example, *Ocimum basilicum* is an important plant that produces essential oils, and its oil has a wide range of chemical structures.[19]

The main objective of this study is to prepare and establish a pseudoternary phase behavior for different extracts of the Palestinian basil (leaves and seeds). In addition to the formation of microemulsions *in-vitro* systems. Also, to proceed with one of the proposed mechanisms to explain the antimalarial effect of the plant, namely the inhibition of beta-hematin formation. Other objectives to be studied and achieved are: Verification of antibacterial activity against five clinical pathogens, antioxidant activity (AA), total phenolic content (TPC), total flavonoid content (TFC), and HPLC chromatography at 254 nm.

Materials and methods

Materials were purchased from Sigma Aldrich: Absolute ethanol (EtOH), methanol, trichloroacetic acid (TCA), phosphate buffer salt solution (PBS), Tween 20, Span 20, ethyl acetate, sodium carbonate, potassium acetate, petroleum ether, distilled water, aluminum chloride ($AlCl_3$), potassium ferricyanide [$K_3Fe(CN)_6$], sodium nitrite ($NaNO_2$), iron standard ($FeSO_4.6H_2O$), sodium acetate, flavonoid standards (quercetin) dimethyl sulfoxide (DMSO) purity 99.5%, nutrient broth, hemin chloride, chloroquine diphosphate salt.

Equipment and apparatus

The following equipment was used in the Laboratory of Chemistry, Pharmacy, Biology and Environmental Sciences at Al-Quds University: Sartorius analytical balance CP, mechanical grinder, Benchtop B4 centrifuge of Jouan, Soxhlet apparatus, rotary evaporator from IKA WEREK RV06- ML, freeze-drying machine (lyophilizes) from Labconco, spectrophotometer (UV2550, Shimadzu, Kyoto, Japan), High performance liquid chromatography (HPLC), StabiliTherm incubator from Thermo.

Methods

Collection and identification of plant materials

The basil plant was collected from the different regions of a cultivated field in Hebron, Palestine, in the summer of 2020. It was identified by Dr. Khalid Sawalha (Assistant Professor of Plant Biotechnology) from the Biology Department of the Faculty of Science, Al-Quds University. This confirmed and identified plant basil is: *Ocimum basilicum L. Lamiaceae = Labiatae*. It is more likely to be this plant with a binomial name. The basil seeds were purchased at a local market in Ramallah, Hebron, and Jerusalem (Figure 1).

Figure 1 *Ocimum basilicum*.

Plant sample

Basil leaves were separated from the stems and then washed with tap water. The cleaned basil leaves were air-dried at room temperature in the shade for about two days, then ground through the mechanical grinder and converted into a coarse powder, and then stored in the refrigerator until use. The seeds of *O. basilicum* were cleaned manually to remove hulls and foreign bodies, and then uniformly ground into a fine powder using a mechanical mill.

Preparation of Basil leaves and seeds ethanolic extract

To identify the chemical constituents of basil, an ethanolic extract was prepared using a Soxhlet apparatus (type FA) in which 15 g of dry powder was placed in 99.5% ethanol for 2 hrs. The ratio of plant powder to solvent was 1:9 (wt./vol) during extraction with the Soxhlet apparatus in 99.5% ethanol. The extract was then filtered after extraction using MN615.110 mm filter paper (Figure 2).

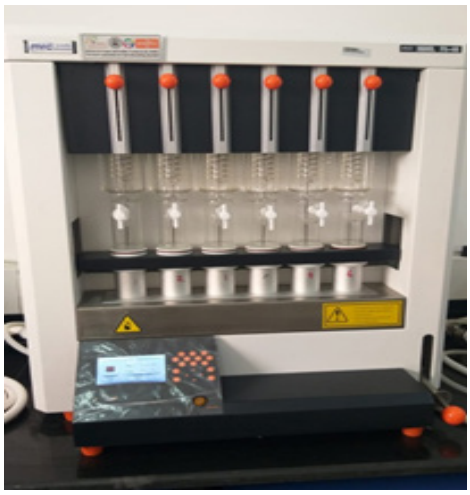

Figure 2 Soxhlet apparatus (model FA).

After filtration, the crude ethanolic extract Basil was concentrated with a rotary evaporator under reduced pressure and lyophilized (freeze-dried) at -40ºC and a pressure of 0.095 mbar until a uniform weight was obtained. On a dry weight basis, the dried powder obtained was 1.2 g (8%), which was determined using the following equation:

$$Percentage\ yield = \frac{Wt\ of\ dried\ extracts \times 100}{Wt\ of\ powder\ taken}$$

The dry extract was stored in an opaque bottle and refrigerated until analysis by HPLC, also for future testing and for further pharmacological studies.[20] The same method was used to prepare the extracts from the leaves and seeds, using different solvents (ethanol

35%, ethyl acetate, and petroleum ether) to perform the antimalarial tests.

Preparation of basil leaves and seeds water extracts

2 g of the basil was soaked in 150 ml of distilled hot water at 90°C for 20 min, then cooled at room temperature and filtered through MN 615 number 110 mm filter paper. Then the excess solvent was gently removed using a rotary evaporator under reduced pressure at 60-70°C to obtain concentrated extracts. The residue was freeze-dried and stored in an opaque bottle until analyzed by HPLC, also for future testing and for further pharmacological studies.

Phytochemical screening for Basil leaves extract

The phytochemical constituents of basil leaves extract were qualitatively studied for the content of phenols, flavonoids, saponins, tannins, alkaloids, triterpenes, and steroids. Basil leaves extract, both freeze-dried and dried, was used for the phytochemical studies.[21]

Phytochemical screening for Basil seeds extract

The phytochemical qualitative reactions of alkaloids, flavonoids, saponins, tannins, and phenolic acids were studied using basil seeds extracts. The color intensity of the resulting precipitate was used as an indicator. The extract of basil seeds, both freeze-dried and dried, was used for the phytochemical tests.[22]

Total phenolic content (Folin–Ciocalteu assay)

Total phenolic content was determined using the Folin-Ciocalteu assay and a spectrophotometric method. Crude basil (leaves/seeds) extracts (0.20 mg) were dissolved in ethanol (99.5%) (20 ml) and 500µl of the solution was diluted in ethanol (99.5%) (10ml). Folin-Ciocalteu reagent (diluted 10-fold; 5 ml, w/v) was added to 1 ml of the sample, followed by sodium carbonate 7.5 percent (5 ml, w/v). The reagents were mixed well, and the mixture was incubated in complete darkness at room temperature for 30 min to complete the reaction. A spectrophotometer (UV2550, Shimadzu, Kyoto, Japan) was then used to read the absorbance of the solution at 269 nm. The calibration curve was prepared using different amounts of gallic acid ranging from 20 to 70 ppm. The results are expressed in milligrams of gallic acid equivalents (milligram GAE per gram dry weight of sample). Measurements were made in triplicate, averaging three replicates. The equation of the standard curve was y = 0.0326x, with a linear regression coefficient (coefficient of determination) of $R^2 = 0.9973$.

Total flavonoid content (TFC)

Aluminum chloride spectrophotometry was used to determine total flavonoids in crude basil extracts. Crude basil extracts (leaves/seeds) (0.20 mg) were dissolved in ethanol (99.5%) (20 ml) and 500µl of the solution was diluted in ethanol (99.5%) (10ml). The extracts (1 ml) were mixed with methanol (3 ml) and $AlCl_3$ solution (0.2 ml, 1:10, w/v), potassium acetate (0.2 ml,0.98:10, w/v) was also added and distilled water (5.6 ml). The reagents were mixed well, and the mixture was incubated in complete darkness for 30 min at room temperature to complete the reaction. A spectrophotometer (UV2550, Shimadzu, Kyoto, Japan) was then used to measure the absorbance of the solution at 420 nm. The calibration curve was prepared using different concentrations of quercetin standard ranging from 10 to 75 ppm. The results were presented as quercetin equivalents in milligrams (milligram QE per gram dry weight of sample). Measurements were made in triplicate, averaging three replicates. The equation of the standard curve was y = 0.0297x, with a linear regression coefficient (coefficient of determination) of $R^2 = 0.9982$.

RP-HPLC analysis of flavonoids

High-performance liquid chromatography (HPLC) is widely used to determine the concentration of flavonoids in natural extracts, both for separation, quantification, and identification of these chemicals. The crude extracts of the two types of plant parts were identified using high-performance liquid chromatography (HPLC), which is an effective tool. Samples of crude extracts (leaves/seeds) at a concentration of 1mg/ ml were prepared by dissolving 10mg of crude extract in 10ml of the appropriate solvent 99 percent ethanol. HPLC with UV detector method was used for quercetin analysis using C18 column (15cm with 4µm particle size) at 254nm wavelength and a mobile phase of water: methanol (50:50 v/v) at a flow rate of 1.0 ml per minutes standard of 0.01 g per 10ml was used.

Phase behavior

The pseudoternary phase diagrams consisting of oil, water, surfactant, and co-surfactant mixture were constructed using the water titration method. The phase behavior of the systems consisting of the ethanolic extract of basil seeds, the water phase [ethanolic extract of basil leaves with ethanol 99.5% as co-surfactant (1:9 (w/v))], and Tween 20 can be represented by a phase tetrahedron, with the peaks representing the pure components. 1g of a combination containing basil oil and Tween 20 in different weight ratios was prepared in screw-capped glass test tubes and shaken through the vortex at room temperature (25°C).

Antibacterial activity

The antibacterial assay was performed using the agar disc diffusion method. Negative controls were prepared with the same solvents (ethanol 99.5%) used to dissolve the samples. The standard antibiotic gentamicin (10µg/disc) and penicillin (10 units) were used as positive controls for the bacteria tested. Antibacterial activity was evaluated by measuring the diameter of the inhibition zones around the disc against the tested bacteria. Clinical isolates of *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Enterobacter sp, and Pseudomonas aeruginosa* were provided by the Health Professions Department, Al-Quds University. Mueller - Hinton Agar was provided by the Department of Microbiology, Faculty of Science and Technology, Al-Quds University.

Measuring the minimum inhibitory concentration (MIC)

MIC is the lowest antimicrobial concentration that inhibits bacterial growth or causes no detectable growth. MIC was determined for all microorganisms tested using the serial broth dilution technique.

A set of four tubes was prepared for each microbe, 2 ml of nutrient broth was added to all tubes, then 2 ml of the aqueous extract of basil (seeds, leaves) (with a concentration of 100 mg/ml) was added to the first tube via a micropipette and mixed thoroughly. Then 2 ml of the solution from the first tube was transferred to the second tube and mixed well. Two milliliters of the solution from the second tube were then transferred to the third tube and then to the fourth tube. After dilution, 200µl of a bacterial suspension standardized to an optical density (0.08-0.1) relative to the McFarland scale (108 CFU/ml) was added to all four tubes. The extract concentration in the first tube was 1000 mg/ml and was diluted three times. Nutrient broth and bacterial cultures were used to prepare positive control tubes. Nutrient broth and solvent were added to the tubes for negative controls. For the bacteria, the tubes were incubated at 37°C for 24 hrs. The clear tubes (showing inhibitory activity) were seen after incubation for each

microbe and the MIC was determined by taking the least clear tube from each set. This test was performed three times for confirmation. All processes were performed under sterile conditions, using a fume hood and an autoclave to sterilize the instruments.

The minimum bactericidal concentration (MBC) which is the minimum concentration that is required to kill the bacteria, had been tested after the results of the MIC. For the bacteria with controls, the tubes of MIC for whole samples (seeds and leaves basil) that indicated no growth (clear) of microorganisms were subcultured in nutrient agar plates and incubated at 37ºC for 24 hrs. The MBC is the concentration of extract that showed no colony growth.

Antioxidant assay

The antioxidant activity of the extracts of basil (leaves and seeds) was determined by a modified method of the ferrous reducing/ antioxidant power assay (FRAP) of Oyaizu M. (1986). Freshly, 0.5 ml of the dissolved sample was mixed with 0.75 ml of phosphate buffer (0.2 M, pH 6.6) and 0.75 ml of a 1% (w/v) potassium ferricyanide solution. Then, 0.75 ml of 10% (w/v) aqueous solution was added to the reaction mixture and incubated at 50°C. The solution of trichloroacetic acid (TCA) was added and the mixture was centrifuged (2000 rpm) for 10 min. Finally, a 0.75 ml aliquot of the centrifuged material was combined with 0.75 ml of H_2O and 0.25ml of 0.1 percent (w/v) aqueous solution of $FeCl_3.6H_2O$. The absorbance at 700 nm was read in comparison to a reagent blank solution containing distilled water, which was also incubated at 50ºC. Aqueous solutions with known Fe (11) concentrations in the range of (2-5 mM) ($FeSO_4.6H_2O$) were used for calibration.[23]

Anti-malarial assay

A semi-quantitative *in-vitro* assay method was used to investigate the potential antimalarial activities of different parts of the Palestinian basil plant (leaves and seeds) using different solvent extracts.

The protocol for evaluating the bio-mineralization of hemin according to Akkawi et al.,[17] consists of incubating a test combination in a typical non-sterile 96-well flat-bottomed plate at 37ºC for 18-24 hrs. The following ingredients were added in the following order: 50µL (0.5 mg/ml hemin chloride) freshly dissolved in dimethyl sulfoxide (DMSO). 100µL of 0.5 M sodium acetate buffer (pH 4.4), followed by 50µL of plant extract or the negative or positive controls. Meanwhile, the mixture was incubated at 37°C for 18-24 hrs in a flat-bottomed plate.

The plate was then centrifuged at 4000rpm for 10min. The pH of the reaction was measured after the supernatant was removed and the final pH was between (5-5.2). To remove free hemin chloride, the remaining pellets were suspended in 200µL of DMSO. The dish was then centrifuged again, and the supernatant was discarded. The β-hematin precipitate was dissolved in 200µL of 0.1 M NaOH to generate alkaline hematin for direct spectroscopic measurement using an ELISA reader at a wavelength of 405nm.

Data presented in Figures and Tables were the average values in some cases three replicates and in another four replicates with standard deviation (SD). The statistical analysis was done for all of the data.

Results and discussion

Phytochemical screening of basil ethanolic extracts (leaves & seeds)

Phytochemical screening of ethanolic basil extracts revealed the presence of medicinally active constituents such as flavonoids, saponins, and tannins:

Total Phenolic Contents (TPC)

Table 1 shows the TPC of basil plant extracts for both leaves and seeds. As can be seen from the table, the TPC of basil extract varies depending on which part of the plant is extracted. The highest TPC was found when the seeds were extracted with 99.5% ethanol, followed by the leaves 99.5% ethanol extract. The results showed that the basil seeds examined in this study contained more phenolic compounds (58.2±0.9 mg/g) than the basil leaves (51.0±2.4 mg/g). Since plant phenolics have multifunctional properties, including the ability to act as singlet oxygen scavengers and scavenge free radicals, the presence of significant amounts of these compounds in Palestinian basil (seeds and leaves) makes the latter an important source of antioxidants that, when consumed properly, may reduce the risk of degenerative diseases, and provide health-promoting benefits. The comparison of the TPC of Palestinian basil with basil from other regions is intriguing. The main phenolic compound content detected in this study, which was extracted with absolute ethanol, was significantly higher than in previous studies (Figures 3-8).[24]

Table 1 Qualitative analysis of Phytochemical constitutes (n =3)

	Phytochemical tested	Test performed	Test Result	
			Leaves extract	Seeds extract
1	Flavonoids	Ethyl acetate test	+	+
2	Saponins	Frothing test	+	+
3	Tannins	ferric chloride test	+	+

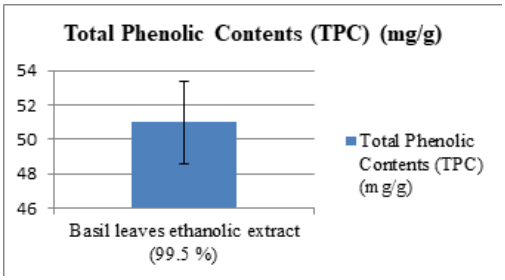

Figure 3 (TPC) (mg/g) for Basil leaves ethanolic extract (99.5 %).

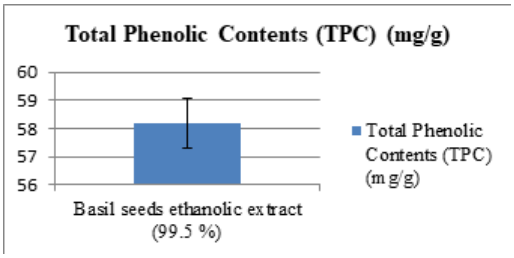

Figure 4 (TPC) (mg/g) for Basil seeds ethanolic extract (99.5 %).

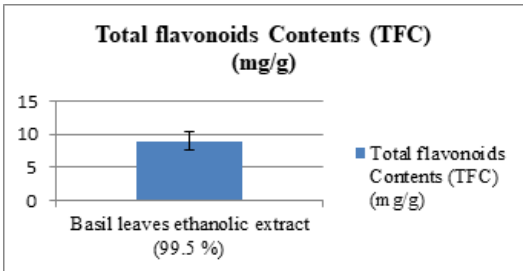

Figure 5 (TFC) (mg/g) for Basil leaves ethanolic extract (99.5 %).

Citation: Tarayrah H, Akkawi M, Yaghmour R, et al. Investigations of the Palestinian medicinal plant basil (*Ocimum basilicum*): antioxidant, antimicrobial activities, and their phase behavior. *Pharm Pharmacol Int J.* 2022;10(3):97–104. DOI: 10.15406/ppij.2022.10.00370

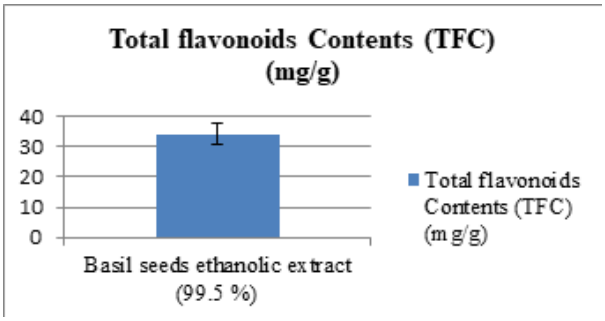

Figure 6 (TFC) (mg/g) for Basil seeds ethanolic extract (99.5 %).

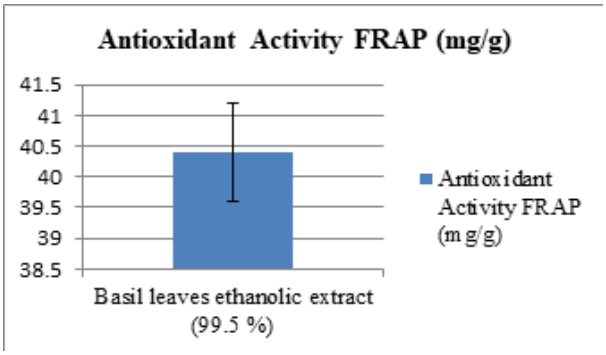

Figure 7 Antioxidant activity FRAP (mg/g DW) for Basil leaves ethanolic extract (99.5 %).

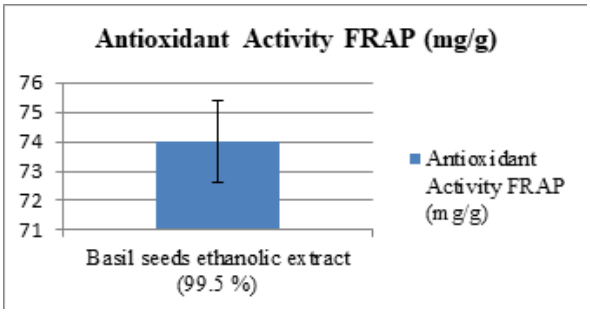

Figure 8 Antioxidant activity FRAP (mg/g DW) for Basil seeds ethanolic extract (99.5 %).

Total Flavonoid Content (TFC)

Table 2 shows the results of the aluminum chloride test for the determination of flavonoid content. The highest TFC was determined for the plant material when the seeds were extracted with 99.5% ethanol, followed by plant material extracted with 99.5% ethanol. The results showed that the basil seeds (34.2±3.6 mg/g) examined in this study contained four times more than the extracted basil leaves (9.0±1.5 mg/g). Comparing the TFC and TPC values of the different parts of the plant, there is a parallelism between the two patterns, with the maximum TPC and TFC concentration found in the extracted basil seeds. Moreover, the TPC value is higher than the corresponding TFC value.

RP-HPLC analysis of flavonoids

The crude extracts were diluted in ethanol at a concentration of 1 mg/ml, injected into a high-performance liquid chromatography (HPLC) and their flavonoids determined. To determine the presence of this chemical in the crude extracts, flavonoid standards were simultaneously injected and separated. Individual standard calibration curves were also prepared at a concentration of 1000 ppm (Figure 9).

Table 2 Total phenolic content (TPC as mg Gallic acid/g DW), total flavonoids contents (TFC as mg Quercetin/g DW), Antioxidant activity FRAP (mg/g DW) (n =3)

	Total Phenolic Contents (TPC) (mg/g)	Total flavonoids Contents (TFC) (mg/g)	Antioxidant Activity FRAP (mg/g)
Basil leaves ethanolic extract (99.5 %)	51.0±2.4	9.0±1.5	40.4±0.8
Basil seeds ethanolic extract (99.5 %)	58.2±0.9	34.2±3.6	74±1.4

An overlay chromatogram of the crude extracts at 254nm is shown in Figure 10. At 6.679 min, the flavonoid chemicals were detected. This wavelength was chosen because the primary peaks near this wavelength had the highest absorbance. The flavonoid molecules were detected at 6.682 min at the same wavelength, as shown in Figure 11.

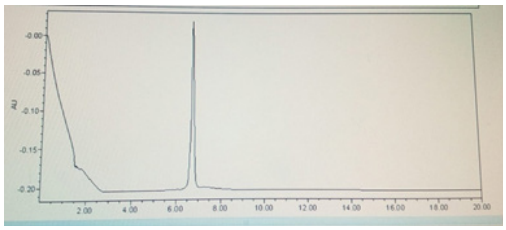

Figure 9 HPLC of standard (Quercetin) at 254 nm.

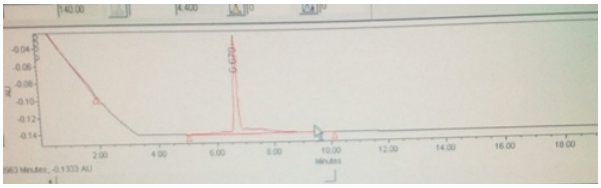

Figure 10 HPLC of basil seed extract showed only one major peak at 254 nm.

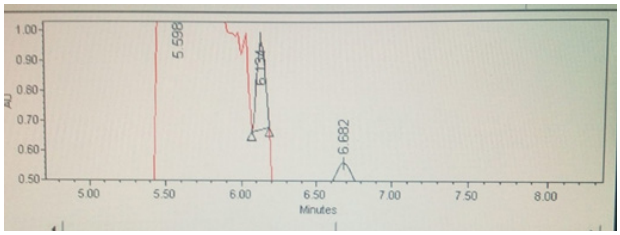

Figure 11 Basil leaves extract high performance liquid chromatography (HPLC) analysis at 254 nm.

Phase behavior

At 25°C, the addition of the water phase to Tween 20 / basil oil in a different ratio system resulted in a ternary phase diagram (Figure 12). The ternary phase behavior of the ethanolic basil extracts was obtained under the same formulation conditions in a serial microemulsion. Two different plant extracts were used, namely [ethanolic basil leaves extract with ethanol 99.5% as co-surfactant (1:9 (w/v))] and ethanolic basil seeds extract at 25ºC.

The microemulsion was identified as transparent by visual inspection after each addition of the water phase. The microemulsion region starts as a single clear isotropic and low viscosity mixture before the addition of the first 9% water from the point containing

20% and less ethanol oil extract and 80% and more Tween 20 and extends to the water peak (100%). Thus, the rest of the phase diagram represents the turbid region based on visual identification. Finally, the phase diagrams were drawn using Origin 2021.

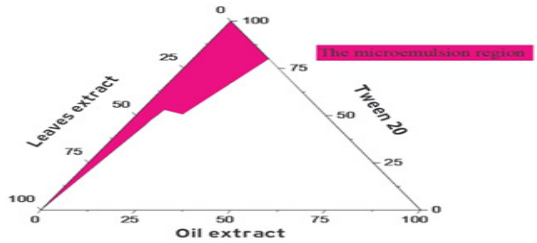

Figure 12 Ternary phase behavior of the Basil ethanolic extracts.

Antibacterial assay

Plants have been used for centuries to treat infections through herbal medicine and self-medication. Especially in Palestine, the use of plants is a long-standing practice based primarily on observation rather than scientific experimentation. However, since the effective life of any antibiotic is limited by bacterial resistance and toxicity, mankind is constantly searching for new antimicrobial drugs. For this reason, the antibacterial activity of *Ocimum basilicum* essential oil against different types of organisms was preliminarily tested in this study.

The antibacterial activity of ethanol extract of *Ocimum basilicum* seeds and leaves were tested against five clinical pathogens (*Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Enterobacter sp, Pseudomonas aeruginosa*). Using the disc diffusion method in the presence of positive controls (gentamicin (10µg/disc) and penicillin (10 units). The zones of inhibition were measured and the average results of the zones of inhibition (Table 3) were summarized (Table 4).

Table 3 The diameter of zone of inhibition of *Ocimum basilicum* seeds against clinical pathogens

Clinical pathogens	Average zone of inhibition (mm)			
	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml
Escherichia coli	ND	ND	ND	8±1.6
Staphylococcus aureus	ND	6±0.8	8±1.7	ND
Klebsiella pneumonia	6±0.2	7±1.4	8±0.8	9±1.7
Enterobacter sp	7±0.8	8±0.9	9±1.8	10±1.2
Pseudomonas aeruginosa	7±0.7	8±0.8	9±1.1	ND

ND: Not detected. Values are given as mean±SD (n =4).

Diameter of inhibition zone (mm) around disc (5 mm).

Standard antibiotic: penicillin (10 units) for *Staphylococcus aureus* and gentamicin (10µg/disc) for the rest.

Comparison between antimicrobial activities (seeds) is illustrated in Figure 13.

Comparison between antimicrobial activities (leaves) is illustrated in Figure 14.

Antimicrobial tests showed that the seeds and leaves extracts had a good zone of inhibition against practically all species. Clinical strains of *Enterobacter sp.* were discovered to be sensitive to the essential oils of basil seeds and leaves (*Ocimum basilicum*) at various dosages. Basil leaves were ineffective against *Klebsiella pneumonia* and *Pseudomonas aeruginosa* at doses ranging from 12.5 mg/ml to 100 mg/ml. The highest zone of inhibition for the seeds extract against *Enterobacter sp* is 10 mm at a concentration of 100 mg/ml, whereas the highest zone of inhibition for the leaves extract against *Enterobacter sp* is 25 mm at a concentration of 100 mg/ml as diffusion (spread).

Table 4 The diameter of zone of inhibition of *Ocimum basilicum* leaves against clinical pathogens

Clinical pathogens	Average zone of inhibition (mm)			
	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml
Escherichia coli	ND	ND	ND	10±0.9
Staphylococcus aureus	12±1.4	13±0.8	14±1.4	18±1.2
Klebsiella pneumonia	ND	ND	ND	ND
Enterobacter sp	13±0.8	16±1.1	18±0.6	25±1.5
Pseudomonas aeruginosa	ND	ND	ND	ND

ND: Not detected. Values are given as mean±SD (n =4).

Diameter of inhibition zone (mm) around disc (5 mm).

Standard antibiotic: penicillin (10 units) for *Staphylococcus aureus* and gentamicin (10µg/disc) for the rest.

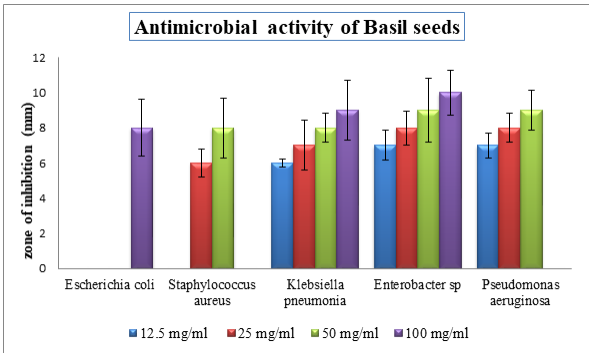

Figure 13 Antimicrobial activity of Basil seeds extracts.

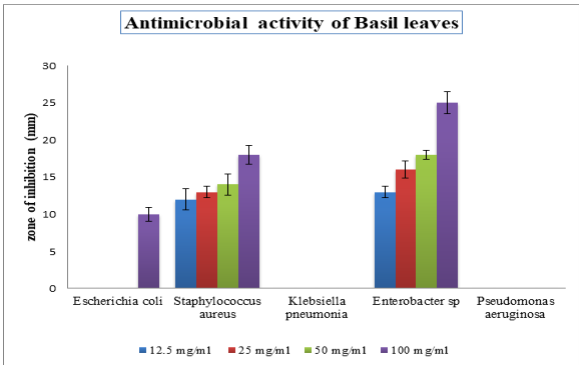

Figure 14 Antimicrobial activity of Basil leaves extracts.

The minimum inhibitory concentration MIC and minimum bactericidal concentration MBC

In this study, MIC and MBC values were determined. Both leaves and seeds were determined as indicated in Table 5. The tubes were tested for turbidity after the dilution technique for the antibiotic (500µg/ml, 250µg/ml, 125µg/ml, 62.5µg/ml) and subsequent incubation, indicating the presence of microorganisms; the organism grows in the negative control tube, which does not contain antimicrobial to prevent growth.

Table 5 The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) of leaves and seeds extract (n=3)

		Minimum inhibitory concentration (MIC) (µg/ml)		Minimum bactericidal concentration (MBC) (µg/ml)	
Microorganism	**Min conc. of extracts (mg/ml)**	**Leaves**	**Seeds**	**Leaves**	**Seeds**
E. coli	100	500	500	>500	>500
Staphylococcus aureus	25	250	62.5	500	125
Pseudomonas	12.5	NT	250	NT	500
Klebsiella	12.5	NT	500	NT	>500

Minimum inhibitory concentration and Minimum bactericidal concentration (values in µg/ml). *NT: Not tested

Because lower amounts of the extract are required to inhibit and kill bacteria, the seed extract was more potent against *Staphylococcus aureus* than another (62.5µg/ml). For *E. coli and Klebsiella*, the seeds have the same MIC (500µg/ml), which is consistent with previous studies. This indicates that their efficacy is equivalent. The concentrations of basil leaves were effective for *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* only from 12.5 mg/ml.

The seeds extract was slightly more potent than the leaves extract, with the MIC for seeds extracts being 62.5µg/ml for *Staphylococcus aureus*, while the MIC for leaves extracts was 250µg/ml. Since 500µg is the maximum concentration used in this study, all values above 500µg/ml require a higher concentration to determine the true value. The MBC results for both seeds and leaves show slightly higher concentration values than the MIC results. For the determination of MIC, the minimum concentration of the extracts was selected, which has a zone of inhibition in the disc diffusion method.

Comparison between minimum inhibitory concentration (MIC) of Basil seeds is illustrated in Figure15.

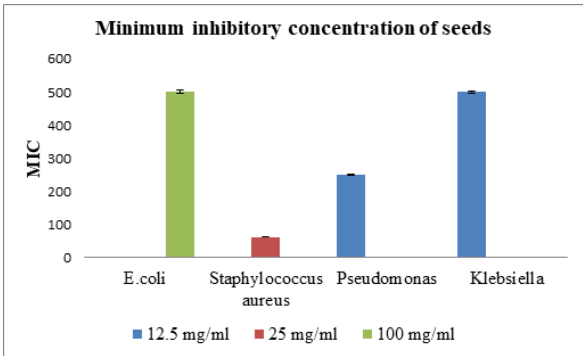

Figure 15 Minimum inhibitory concentration of Basil seeds.

Comparison between MIC of Basil leaves is illustrated in Figure 16.

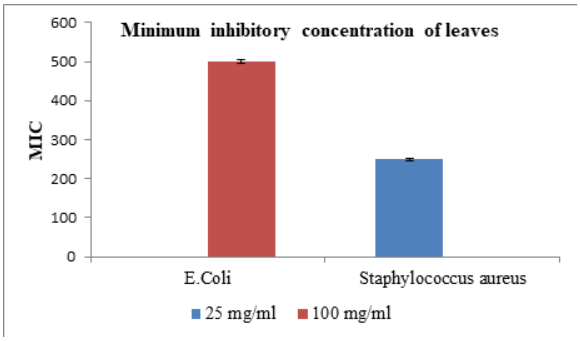

Figure 16 Minimum inhibitory concentration of Basil leaves.

Antioxidant assay

The FRAP assay was used to determine antioxidant activity. This assay indicates the electron donor capacity of the tested compounds by measuring the ability of the substance to reduce Fe^{+3} to Fe^{+2} ions. At 700 nm, the Fe^{+2} ion is detected spectrophotometrically by determining its colored compound with ($FeSO_4.6H_2O$).

Table 2 shows the antioxidant activity of both the seeds and leaves of the Palestinian basil plant. As can be seen from the data, the extraction of the seeds of the plant contains 74±1.4 mg/g. Compared with the antioxidant activity of the leaves part 40.4±0.8 mg/g, the portion of the Palestinian seeds in this study has a higher antioxidant activity than the leaves. This result is in perfect agreement with the total phenolic content calculated as milligrams of gallic acid per gram of dry weight (DW) of the basil plant.

Antimalarial assay

It has been suggested that there is more than one mechanism explaining the potential *in-vitro* antimalarial effect of the alcoholic (ethanolic) extract of *Ocimum* species, and this should be further investigated.[19] The intraerythrocytic stage of the parasite life cycle is a potential target for antimalarial drugs. During this stage, Plasmodium parasites reside inside the host's erythrocytes and degrade hemoglobin, resulting in the accumulation of free heme; ferriprotoporphyrin (IX), which is toxic to the parasite and can produce oxygen radicals. The heme is detoxified by being incorporated into an insoluble crystal known as hemozoin or malarial pigment.

Purified hemozoin, a polymer used for *in-vitro* studies in antimalarial drug research, is structurally, chemically, and spectroscopically identical to β-hematin, a synthetic polymer made from ferriprotoporphyrin- IX.

In the present study, extracts of basil leaves, and seeds prepared with different solvents (absolute ethanol, ethanol 35%, ethyl acetate, and water) were evaluated for their potential inhibitory effect on beta-hematin formation using a semi-quantitative *in-vitro* method according to Akkawi et al.[17] The efficiency of these extracts in inhibiting β-hematin formation *in-vitro* was compared with positive (CQ -chloroquine 0.1 mg/ml) and negative controls (water) at 405nm. It was found that the extracts of Palestinian basil (*Ocimum*) used as anti-malarial agents did not inhibit the formation of β-hematin under certain chemical and physiochemical conditions, thus excluding one of these possible mechanisms.

Conclusion

The chemical constituents of basil from different sites in Palestine and some of its pharmacological effects were studied. The pseudoternary phase diagram was determined at room temperature. A basil microemulsion was successfully prepared by combining basil

leaves and seeds extract with Tween 20 as a surfactant. The antioxidant activity of sweet basil was largely associated with flavonoids, followed by phenolic acids. Seeds were found to have a higher concentration of phenolic and flavonoid components in the extracts than leaves. HPLC technique was used and proved to be precise, accurate, and reliable in identifying the flavonoid components of Basil Palestrina. The antibacterial activity of basil essential oils against resistant clinical strains was demonstrated in these studies. Moreover, the basil oil (seeds) tested was more effective against all clinical strains than the basil leaves extract. Thus, the action of essential oils against bacteria with different resistance mechanisms could be effective not only in treating but also in preventing the spread of resistant strains. Finally, under certain chemical and physiochemical conditions, the activity of Palestinian basil herb (*Ocimum*) as an antimalarial agent that does not interfere with the synthesis of β-hematin was determined *in-vitro.*

Acknowledgments

The authors would like to express their great gratitude to Dr. Mohammad Abul Haj, Dean of the Faculty of Science and Technology, and Mr. Sameh Nuseibeh, technician and researcher in the Laboratory of Agricultural Research and Analysis, who did not hesitate for a moment to help and support.

Conflicts of interest

Authors declare that there is no conflict of interest.

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