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Original Article

Medicinal Applications, Chemical Compositions, and Biological Effects of an Algerian *Ocimum basilicum* L.var *Genovese*; with the Conversion of Experimental Doses to Humans

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Abstract

Introduction: This paper aims to analyze the medicinal uses of *Ocimum basilicum* L.var *Genovese* (basil) in western Algeria and its effectiveness.

Materials and Methods: For the experiments, 154 structured questionnaires were collected to list the medicinal uses of basil. The essential oil of *O. basilicum* (EOB) obtained by hydro-distillation was analyzed by the GC/MS. The ethanolic and aqueous extracts (EEB and AEB) were analyzed by HPLC. The antioxidant activity was measured by DPPH assays and the antimicrobial activity was measured against five microbes. For the in vivo study, *Swiss albinos* mice were used to determine the toxicity using Lorke's method. The anti-inflammatory activity was determined using the Carrageenan method. The experimental doses were converted from mice to humans using the K_m factor.

Results: The ethnobotanical study indicates that local people use basil to treat diseases and health problems (50% for inflammation and 38.11% for microbial diseases). The results also show that EOB contains 41.3% linalool, whereas ethanolic extract contains benzoic acid (50.86 mg/g). The IC_{50} value is 556, 878.7, and 962.3 µg/ml for EOB, EEB, and AEB, respectively. The EOB and AEB inhibit the positive Gram bacteria and yeast; the EEA inhibits the negative Gram. The LD_{50} is 400, 470, and >5000 mg/kg for AEB, EOB, and EEB respectively. The results of the anti-inflammatory test highlight 76.33, 71.0, and 60.43% inhibition of edema at a 100 mg/kg dose for EOB, AEB, and EEB, respectively.

Conclusions: The Algerian basil can be considered as an antioxidant, antimicrobial and anti-inflammatory.

Keywords: Anti-inflammatory, Antimicrobial, Basil, Ethnobotany, Human Dose, Toxicity

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Introduction

In Algeria, *Ocimum basilicum* L.var *Genovese* (Lamiaceae) is frequently used in cooking and medicinal purposes. Therefore, studying the biological and pharmacological properties of the extracts of this plant is an effective approach in searching for new drugs to valorize the basil cultivated in western Algeria. Various vigorous effects characterize Basil, including antimicrobial, antifungal, insecticidal, antiparasitic, antioxidant, immunomodulatory, anti-inflammatory, hepatoprotective, anti-osteoporotic, cardioprotective, neuroprotective, and anti-cancer.[1] Sweet basil is an aromatic herb that has been utilized in traditional medicine. The basil contains volatile oil,[2] caffeic acid derivatives, and flavonoids responsible for the drug's antimicrobial, diuretic, and digestive stimulant properties. Essential Oils (EOs) are natural compounds formed by various aromatic plants and are secondary metabolites.[3] Major aromatic compounds from volatile oils of basil manifest an anti-oxidative activity. The major compound of *O. basilicum* is linalool (35.1%).[4] The latter has an anti-inflammatory effect in different animal models.[5]

Many studies have analyzed the oil and extracts of *O. basilicum* L.var *Genovese*. However, no in-depth study on its EO and extracts has been conducted so far in western Algeria. This study aims to provide insightful data about the plant's indications, treatment, posology, and chemical composition by analyzing its EO and extracts. The biological phenomenon was experimentally investigated based on interrogation. Most of the basil-treated symptoms are correlated

with inflammation, microbial infection, or the accumulation of oxidants in the organism. Thus, the antimicrobial activity was tested against five selected microbial strains causing the microbial disease indicated in the ethnobotanical study.

For the in vivo study of toxicity and anti-inflammatory activity of EOs, ethanolic and aqueous extracts were extracted from the Algerian basil for consistent results. It should be mentioned that according to the literature, dose extrapolation by simple conversion based on body weight alone is not a viable method. A miscalculation in dose conversion may result in adverse effects due to overdosing or reduced potency due to underdosing. Therefore, in this study, the conversion of experimental doses from mice to humans was based on body weight and BSA as described in the FDA draft guidance.[6]

Materials and Methods

Data Collection

A questionnaire was used to obtain the data and knowledge of traditional basil therapists in western Algeria to treat diseases. This ethnobotanical study was conducted from 2017 to 2019, with 154 informants interviewed using a free list and semi-structured interviews. First, a questionnaire was prepared and completed during a face-to-face interview. The questionnaire included demographic information (location, interviewer characteristics, and education level). In addition, it included facts about the plant and its use (native name, used organ(s) of the plant, and recipe). Thus, the interviewees spoke to the respondents in person and received their pharmaceutical and ethnobotanical information. Then, the information was recorded in the questionnaires. The data were finally analyzed using the Excel software.[7]

Collection of Samples and Extraction

After confirmation by botanical experts, the aerial part of the Algerian basil variety, namely *Ocimum basilicum* L.var *Genovese*, was harvested in July 2018. The cuts were made at the beginning of basil flowering in Ain Temouchent, in western Algeria. Then, it was washed with distilled water and dried for 21 days. Finally, it was ground into powder.

Essential Oil Extraction

Hydro-distillations were performed on a circulatory Clevenger-type apparatus according to the procedure described in the European Pharmacopoeia.[8] The EOB was separated, measured, and stored in an airtight glass vial covered with aluminum foil at 4-5 °C until the analysis stage.

Ethanolic Extract (EEB)

A quantity of 10 g of powder from aerial parts of the plant was extracted with 80% ethanol (1:10 w/v) and macerated for 24 h. After that, the product was filtered and concentrated with rotavapor.[9]

Aqueous Extract (AEB)

The aqueous extract was prepared by decoction: 50 g of the powdered plant was boiled in 500 ml distilled water for 20 min at 80 °C (at a 1:10 w/v sample to solvent ratio). Then, it was filtered and lyophilized to obtain a dark brown powder. Each dry extract was weighed then the yield was calculated and stored at 4 °C.[10]

GC-SM Analysis

Analysis of the EOB was realized by GC-MS software. The software was adapted to handle mass spectra and chromatograms in the ChemStation.[11] Mass spectra libraries were used as references. Specimens were dipped in chloroform with a dilution rate of 1:100. Also, compounds were identified by asserting their mass spectra and retention times with those mentioned in the literature. The percentage of each component was calculated based on GC peak areas. The response factors were estimated using standard compounds having the same molecular weight as the compound families that constitute the EO (hydrocarbon monoterpenes, oxygenated monoterpenes, hydrocarbon sesquiterpenes, and oxygenated sesquiterpenes).[12]

Analyses of Phenolic Compounds (HPLC)

The phenolic composition analyses of different extracts were made according to the Caponio, Alloggio[13] approach, yet with slight modifications. The performance was assessed using an HP-Agilent 1290 Infinity HPLC equipped with a C18 column and diode array detector DAD. As a mobile phase, 3% acetic acid in (A) water and methanol (B) were used. Injection volumes were 1 µl, and extract concentrations were 20 mg/ml. The eluates were detected at 278 nm. The samples were prepared in methanol. Finally, injection volumes were 20 µl. The elution gradient applied at a flow rate of 0.8 ml/ min was: 93% A-7% B (0.1 min), 72% A-28% B (20 min), 75% A-25% B (8 min), 70% A-30% B (7 min) and the same gradient for 15 min was 67% A-33% B (10 min), 58% A-42% B (2 min), 50% A-50% B (8 min), 30% A-70% B (3 min), 20% A-80% B (2 min) and 100% B in 5 min until the end of the experimental cycle. Gallic acid, catechin, chlorogenic acid, caffeic acid, hydroxybenzoic acid, Epicatechin, syringic acid, coumaric acid, trans-ferulic, sinapic acid, benzoic acid, acid hesperidin, rosmarinic acid, cinnamic acid, and quercetin were used as standards. Identification and quantitative analysis were made by comparison with standards. The amount of each phenolic compound was expressed as mg per gram of extract using external calibration curves obtained for each phenolic standard.[14]

Antioxidant Activity Determination

The antioxidant activity was determined using the DPPH method described by Mansouri et al.[15] A duplicate reading was performed for each concentration. Ascorbic acid was

used as a positive control. The radical scavenging activity was calculated, and IC_{50} values were determined graphically from the sigmoidal-shaped curve of antioxidant concentration (µg/ml) versus inhibition (%). For comparison purposes, the reciprocal $1/IC_{50}$ values were used.

Antimicrobial Activity Assay and Serial Microdilution Assay

Bacteria and Growth Conditions

Five microorganism species were employed as test organisms: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, *Bacillus cereus* ATCC 6633, and *Candida albicans* ATCC 10231 (Laboratory for Research on Local Animal Products, Ibn Khaldoun, Tiaret, Algeria). The strains were deposited in Eppendorf tubes, each containing a bacterial culture preserved in nutrient broth supplemented with 30% glycerol and maintained at -20 °C. A volume of 100 µl of each tube was transferred into the Brain Heart Infusion (BHI) broth and then incubated at 37 °C. The referenced strains were developed after 24 to 48 h, indicating their reactivation.

Antibiogram and Aromatogram

The microbes' selection was determined based on the pathogens that caused the diseases mentioned in the ethnobotanical study. Moreover, antibacterial activity was determined by the agar disc diffusion assay.[16] Further, the extracts were dissolved in dimethyl sulfoxide (DMSO 10%). Subsequently, the inoculated surface of petri plates was prepared with Mueller Hinton (MH agar). Finally, sterile filter paper discs (6 mm in diameter) were impregnated with 20 µl of the extract solution. Erythromycin 15 µg (E), Penicillin 10 µg (P) Amoxicillin 10 µg (AM) Colistin 10 µg and Pristinamycin 15 µg were used as positive controls. Negative controls were performed using paper discs loaded with 20 µl of the used solvents (DMSO 10%). After incubation at 37 °C for 24 h, the diameter of inhibition zones was measured in millimeters through the agency of Vernier calipers. All tests were carried out in triplicate to ensure reliability. An inhibition zone of 14 mm or greater (including the disc's diameter) was considered a high antibacterial activity.[17]

Minimal Inhibitory and Minimal Bactericidal Concentrations (MIC and MBC)

MIC and MBC were determined by broth microdilution assay[18] using a 96-well polypropylene plate and Mueller-Hinton broth. After 24 h at 37 °C, MIC was considered the lowest substance concentration that prevented visible bacterial growth in the well. MBC was defined as the lowest concentration yielding negative subcultures. For 24 h, a 10 µl aliquot from each well that showed no bacterial growth was sub-cultured into MH agar. The experiment was carried out in triplicate. The MBC/MIC report of extract provides information on the antimicrobial power. Indeed, when this ratio is less than or equal to 4, the extract is said to be bactericidal, while if it is greater than 4, it is said to be bacteriostatic.

Test Acute Toxicity

All experimental protocols were prepared and performed based on the ethical guidelines of the Institutional Animal Ethical Committee. *Swiss albino* mice (25-30 g) were used following Lorke's method.[19] The animals were controlled every 10 min and were then respectively kept under observation for 24, 48, and 72 h to identify potential changes correlated to motor activity, respiration, writhing, and piloerection.[20]

Anti-inflammatory Activity

Carrageenan (1%) induced hind paw edema model, which was used to determine anti-inflammatory activity.[21] Furthermore, the animals were divided into five groups (n = 6). The administration mode used was as an intraperitoneal injection. The first group was treated with saline 0.9% (control group), while five groups were treated with AEB and EEB (100 mg/kg) and the EOB with three doses (100, 50, and 10 mg/kg) (experimental groups). Moreover, Diclofenac (10 mg/kg) was used as the reference drug. Paw edema was then measured every 60 min for 6 h after the induction of inflammation. The difference in foot-pad thickness was measured by a gauge caliper (FISCHER DAREX). The mean values of treated groups were compared with those of a control group, and statistical analyses were performed.[22]

Dose Conversion (Mice to Humans)

The developed calculator uses the K_m ratio of different animal species based on the draft FDA guidelines[5] and calculates the dose between species with the factor K_m as the unit of scale, as follows:

Human equivalent dose (mg/kg) = Dose to be converted/ (Human K_m/Mouse K_m) (1)

The formula used to calculate the dose is based on the K_m ratio of different species. The conversion of dose per kg of body weight between species and dosage can also be calculated for the animal weight (according to the experiments).[23]

Statistical Analysis

The data are presented as the mean ± Standard Error of the Mean (SEM) or mean ± Standard Deviation (SD). In the anti-inflammatory activity, the differences between the means were evaluated by analysis of variance (ANOVA), followed by Dunnet's test. Statistical differences were considered significant at $p<0.05$.

Table 1. Therapeutic Internal Uses Recorded

Internal Uses			
Indications	**N**	**%**	**Recipe**
Analgesic	13	8.44	Drink one glass of Basil herbal tea during pain
To remove sputum	11	7.14	Drink a mixture of one cup of basil juice, one teaspoon of ginger, and one teaspoon of honey.
Tonsils	10	6.49	Drink the infusion of a spoonful of basil in a cup of water two times a day Chew a small amount of basil every day.
Nausea	9	5.84	Mix a teaspoon of natural honey and ground ginger in a glass of basil juice, and drink the mixture twice a day
Flu and colds	9	5.84	Drink a glass of basil decoction daily.
Antipyretic	8	5.19	Drink soaked basil twice a day.
Diarrhea	7	4.54	Take a glass of Basil herbal tea before sleeping.
Kidney stones	7	4.54	Mix a teaspoon of honey and a glass of basil juice, and drink twice a day.
Insomnia	6	3.89	Take a glass of Basil herbal tea before sleeping.
Bad breath	6	3.89	Chew basil freshly or gargle daily. Chew a small amount of basil every day.
Appetite	6	3.89	Drink half a glass of basil herbal tea before the meal. Add fresh basil to the meal.
Atherosclerosis Hypertension	5	3.24	Take a glass of basil herbal tea before sleeping.
Bronchitis	5	3.24	Mixa teaspoon of ginger and honey in a glass of basil juice, and drink the mixture once a day
Immunity booster	5	3.24	Mix a teaspoon of white honey, one cup of basil juice, and a slice of turmeric twice a week.
Soothing	5	3.24	Drink a glass of basil herbal tea
Intestinal worms	4	2.59	Drink a glass of Basil herbal tea in the morning and before sleeping
Fatigue and stress	3	1.94	Drink a glass of basil herbal tea to ease fatigue or stress
Cancer	3	1.94	Drink a glass of Basil herbal tea before sleeping. Eat fresh basil leaves with meals.
Stomachache	3	1.94	Add a teaspoon of ground basil to a glass of water, mix and then drink the mixture twice a day.
Vomiting	3	1.94	Drink a glass of basil decoction daily
Skinny	2	1.3	Mix a teaspoon of honey, one teaspoon of ginger, and one cup of basil juice. Drink them in the morning before eating.
Sweating	2	1.3	Drink one glass of Basil herbal tea before physical effort
Total	132	85.71	

N: Number of Responses, %: Percentage of Responses.

Table 2. Therapeutic External Uses Recorded

External Uses			
Indications	**N**	**%**	**Recipe**
Rheumatism	3	1.94	Add basil powder to hot oil, leave the mixture for 5min, massage the skin, and cover the massaged area.
Anti-hair loss	2	1.3	Mix half a cup of finely chopped basil and a cup of castor oil, then apply the mixture to the hair, leave for eight hours, and wash it off with water.
Headaches	2	1.3	Aspirate the vapor from the infusion of basil.
Skin diseases	2	1.3	Mix three tablespoons of basil, one tablespoon of sesame oil, and one cup of boiling water. Use the filtrate on the skin
Migraine and sinusitis	2	١,٣	Take a small amount of basil with two cups of boiling water, cover the head with a towel, and aspirate.
Hemorrhoids	2	1.3	Crush the fresh basil leaves and put them in a compress on the area overnight.
Mood improving	1	0.65	Take a glass of basil herbal tea
Dandruff and fungus	1	0.65	Apply a mixture of one tablespoon of ground basil and three tablespoons of coconut oil to the hair. massage it for 10 minutes, then wash the hair with water
Blackheads	1	0.65	Make a mask with a mixture of a tablespoon of ground basil and egg white. Massage the face well. Leave for 30 min, rub and rinse with warm water.
Gingivitis	1	0.65	Mix a teaspoon of mustard oil and finely chopped basil. Apply the mixture to the teeth, leave it for five minutes, then wash it off with water.
Gray hair	1	0.65	Mix a cup of fine basil, one cup of castor oil, and three tablespoons of coconut oil, and put it on the hair for 8 hours.
Muscle spasms	1	0.65	Mix the basil powder and olive oil; Leave the mixture for 12 hours, then massage the area.
Cough	1	0.65	Aspirate the vapor from the infusion of basil.
Angina	1	0.65	Crush the fresh basil leaves on the throat from the outside, and cover for 2 hours.
Skin wounds	1	0.65	Apply basil juice to the wound 4 times a day.
Total	22	14.29	

N: Number of Responses, %: Percentage of Responses.

Results

Ethnobotanical Study

The results of the completed questionnaire indicate that out of 154 people who use basil to treat diseases, 26.6% have a medium level of education, 36.36% are single, 22.07% of them are between 20 and 30 years old, 74% are women, 34.41% have no monthly salary; 57% use basil from the garden, 22.22% receive information according to their herbalist, 85.7% of the treated diseases are internal diseases, 80% are non-chronic diseases, 42% use basil for digestive

Table 3. Chemical Composition of *Ocimum basilicum* Essential Oil from Algeria

No	*RT*	I_k	Compound	Content, %	Formula	Class
1.	7.709	1026	Ortho-cymene	0.4	$C_{10}H_{14}$	MH
2.	7.918	1031	1,8-cineole	5.2	$C_{10}H_{18}O$	MO
3.	8.482	1050	*(E)-B*-ocimene	0.8	$C_{10}H_{16}$	MH
4.	9.134	1070	*Cis*-sabinene hydrate	0.6	$C_{10}H_{18}O$	MO
5.	10.340	1096	Linalool	41.3	$C_{10}H_{18}O$	MO
6.	13.339	1177	terpinen-4-ol	4.1	$C_{10}H_{18}O$	MO
7.	13.886	1188	α– terpineol	2.9	$C_{10}H_{18}O$	MO
8.	16.631	1256	Trans-sabinene hydrate acetate	2.1	$C_{12}H_{20}O_2$	MO
9.	20.504	1349	α– terpynil acetate	3.2	$C_{12}H_{20}O_2$	MO
10.	20.904	1389	eugenol	0.3	$C_{12}H_{20}O_2$	MO
11.	22.812	1403	Methyl eugenol	1.3	$C_{11}H_{14}O_2$	MO
12.	23.324	1419	(*E*)-caryophyllene	1.1	$C_{15}H_{24}$	SH
13.	24.128	1439	α– guaiene	1.0	$C_{15}H_{24}$	SH
14.	24.688	1454	α-Humulиne	1.5	$C_{15}H_{24}$	SH
15.	24.928	1456	*(E)-β*-farnйsиne	0.6	$C_{15}H_{24}$	SH
16.	25.802	1485	germacrene *D*	3.4	$C_{15}H_{24}$	SH
17.	26.419	1500	bicyclogermacrene	1.9	$C_{15}H_{24}$	SH
18.	26.755	1509	α-bulnesene	2.1	$C_{15}H_{24}$	SH
19.	27.118	1513	γ-cadinene	2.5	$C_{15}H_{24}$	SH
20.	29.527	1578	spathulenol	6.0	$C_{15}H_{247}O$	SO
21.	30.961	1619	1,10di-lpi-cubinol	1.2	$C_{15}H_{26}O$	SO
22.	31.923	1637	β-acorenol	10.3	$C_{15}H_{26}O$	SO
23.	32.233	1650	β-eudesmol	0.5	$C_{15}H_{26}O$	SO
24.	32.417	1653	α-eudesmol	1.2	$C_{15}H_{26}O$	SO
Total identified				95.4 %		
Oxygenated monoterpenes (MO)				62%		
Sesquiterpene oxygen (SO)				19.2 %		
Hydrocarbon sesquiterpene (SH)				13%		
Hydrocarbon monoterpenes (MH)				1.2 %		

RT: Retention Time, I_k Kovats Index.

Table 4. The Polyphenolic Compounds Content in the Studied Species

	EEB				AEB			
Pk	Compounds	*C*, mg/g	Content, %	*RT*/min	Compounds	*C*, mg/g	Content, %	*RT*/min
1	Benzoic acid	50.8	23.048	37.35	Hydroxybenzoic acid	1.20	32.622	4.296
2	Trans-ferulic acid	2.08	18.043	3.952	Benzoic acid	0.81	18.243	23.35
3	Catechin	0.32	13.539	4.353	Catechin	0.59	17.847	38.56
4	Epicatechin	0.31	10.127	4.815	Trans-ferulic acid	0.45	4.246	79.92
5	Hydroxybenzoic acid	0.28	08.601	25.36	Epicatechin	0.38	7.126	7.422
6	Hesperidin acid	0.27	07.201	7.253	Sinapic acid	0.25	3.494	77.01
7	Coumaric acid	0.26	5.043	14.18	Chlorogenic acid	0.20	3.235	6.300
8	Sinapic acid	0.20	3.417	79.79	Hesperidin acid	0.19	3.197	9.241
9	Rosmarinic acid	0.18	2.133	10.10	Quercetin	0.17	3.150	66.07
10	Chlorogenic acid	0.09	1.911	3.559	Gallic acid	0.15	3.092	79.08
11	Gallic acid	0.08	1.873	77.25	Syringic acid	0.14	1.332	78.42
12	Cinnamic acid	0.04	1.722	3.385	Rosmarinic acid	0.13	1.317	77.59
13	Quercetin	0.03	1.710	10.76	Cinnamic acid	0.10	1.039	75.42
14	Syringic acid	0.02	1.625	6.192	Coumaric acid	nd	-	-
15	Caffeic acid	nd	-	-	Caffeic acid	nd	-	-

nd (-): not determined, below limit of detection; Pk: peak; C: concentration mg/g extract; RT: Retention time; EEB: Ethanolic extract of *O. basilicum*; AEB: Aqueous extract of *O. basilicum.*

diseases, 29% use basil in decoction, 42.2% use it orally, 63% use the aerial part of basil for medicine, 84.4% keep it away from moisture, 60.38% only use it for a few days, 35.06% find it very effective, and 51.29% think it has side effects. Therefore, pregnant women and children should avoid it. Table 1 lists 23 diseases treated by *O. basilicum* with an internal application, and Table 2 summarizes 15 diseases treated by *O. basilicum* with an external application.

Yield

EOB yield was 1.36% with a yellowish-green color, while EEA and AEB were 19.63 and 23.45%, respectively.

GC/SM Analysis

GC/SM of EOB extracted by Clevenger manifested 29 compounds; 24 were determined (95.4%). The major compound is Linalool (41.3%) and B-Acorenol (10.3%). Table 3 lists the EOB composition in the percentage of chromatographic peak areas. Oxygenated Monoterpenes (MO) represent (62%) of the EOB.

Analyses of Phenolic Compounds (HPLC)

Table 4 summarizes the phenolic compositions of *O. Basilicum* extracts. For each extract, 14 phenolic compounds (Gallic acid, Catechin, Chlorogenic acid, Caffeic acid, Hydroxybenzoic

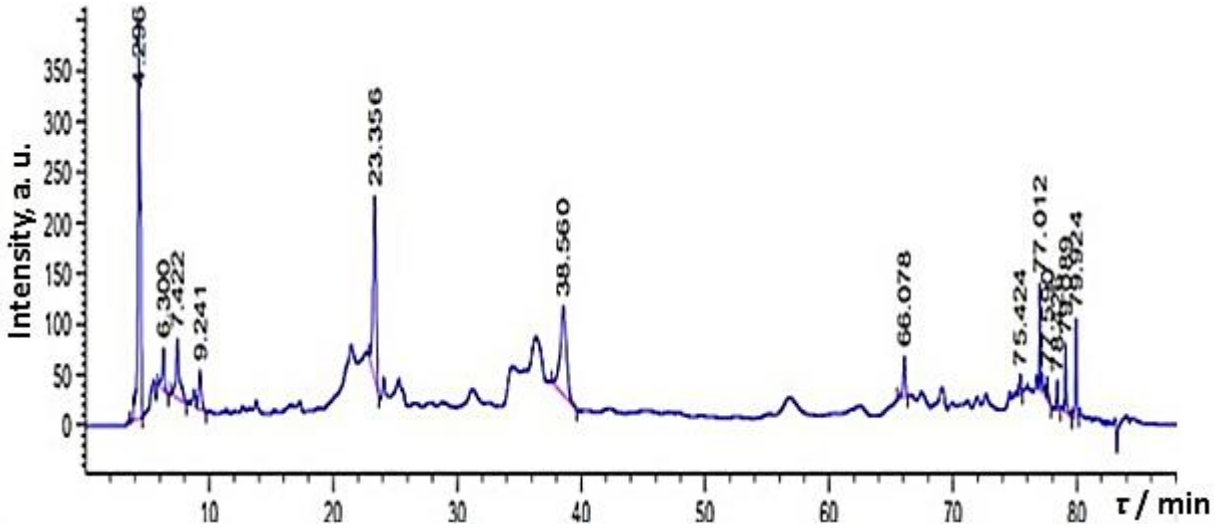

Figure 1. HPLC Chromatogram of Ethanolic Extract of *O. basilicum* (EEB).

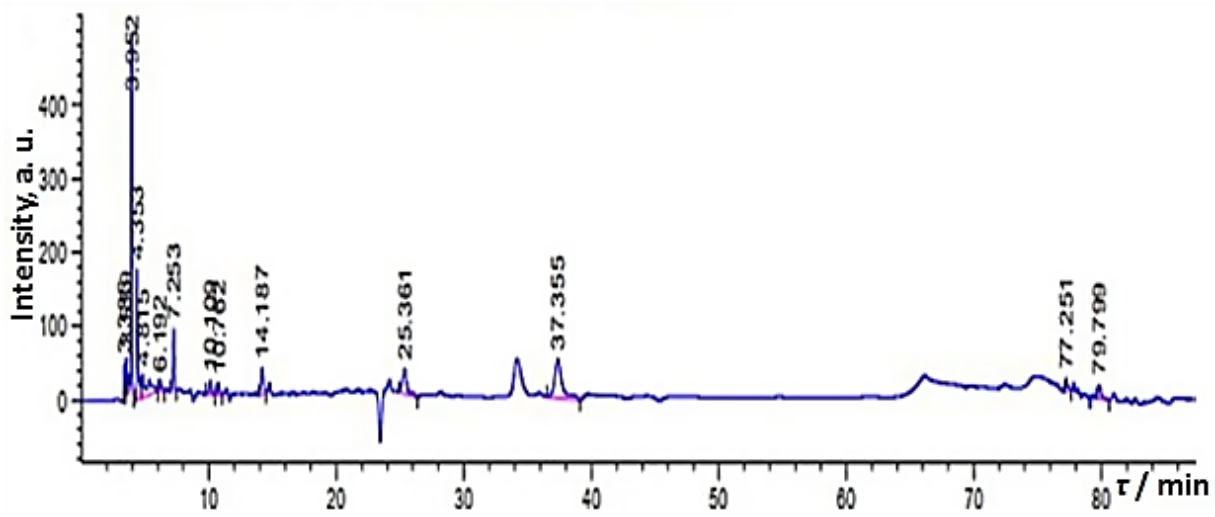

Figure 2. HPLC Chromatogram of Aqueous Extract of *O. basilicum* (AEB).

Hydroxybenzoic acid, Epicatechin, Syringic acid, Coumaric acid, Trans-ferulic acid, Sinapic acid, Benzoic acid, Hesperidin, Rosmarinic acid, Cinnamic acid, and Quercetin) were investigated. Figures 1 and 2 represent the chromatograms. In the ethanolic extract, 14 components were determined. It can be noted that Benzoic acid is the most abundant compound in this extract (50.86 mg/g), while Trans-ferulic acid is 2.08 mg/g. The other compounds show a concentration of less than 1 mg/g. The extract did not contain Caffeic acid.

For the aqueous extract, 13 compounds were identified, except the Coumaric acid and Caffeic acid. It can be noted that the Hydroxybenzoic acid has the highest level (1.2 mg/g). All other compounds have a concentration of less than 1 mg/g. Moreover, the results indicate that Benzoic acid, Catechin, Trans-ferulic acid, Epicatechin, and Sinapic acid are dosed with 0.81, 0.59, 0.45, 0.38, and 0.25 mg/g extract, respectively.

Antioxidant Activity Determination

The obtained results using the DPPH Method indicate a considerable diversity of the capacity to scavenge free radicals between EOB and ascorbic acid. The EOB exhibits DPPH scavenging capacity dependently with IC_{50} of (556 µg/ml) and (R^2 = 0.8919). Its antioxidant activity is less effective than that of the reference substance. The ascorbic acid shows the strongest DPPH scavenging capacity with IC_{50} of (402 µg/ml and (R^2 = 0.9973). Table 5 lists the rapport ($1/IC_{50}$). Considerable diversity in the capacity to scavenge free radicals between oil and extracts of this plant (Table 5) can be observed. EEB shows moderate scavenging effects against DPPH radical (78.14%) compared with AEB (90.84%) at 2 mg/ml.

Antibacterial Activity Assay

Antibiogram Test

Figure 3 shows that *E. coli* is very sensitive to Erythromycin

Table 5. IC_{50} and 1/ IC_{50} Values Obtained in the DPPH Radical Scavenging Assay

	IC_{50}, µg/ml	$1/IC_{50}$, µg/ml^{-1}	RSA, %	R^2	Y
EOB	556	0.0017	88.42	0.8919	0.4317x+47.600
EEB	878.73	0.0011	78.14	0.9780	0.2775x+25.615
AEB	962.35	0.0010	90.84	0.9958	0.4027x+11.246
Ascorbic Acid	402	0.002	98.39	0.9973	121.73x-1.0356

RSA: Radical Scavenging Activity at 2 mg/ml of Plant Extracts and Ascorbic Acid.

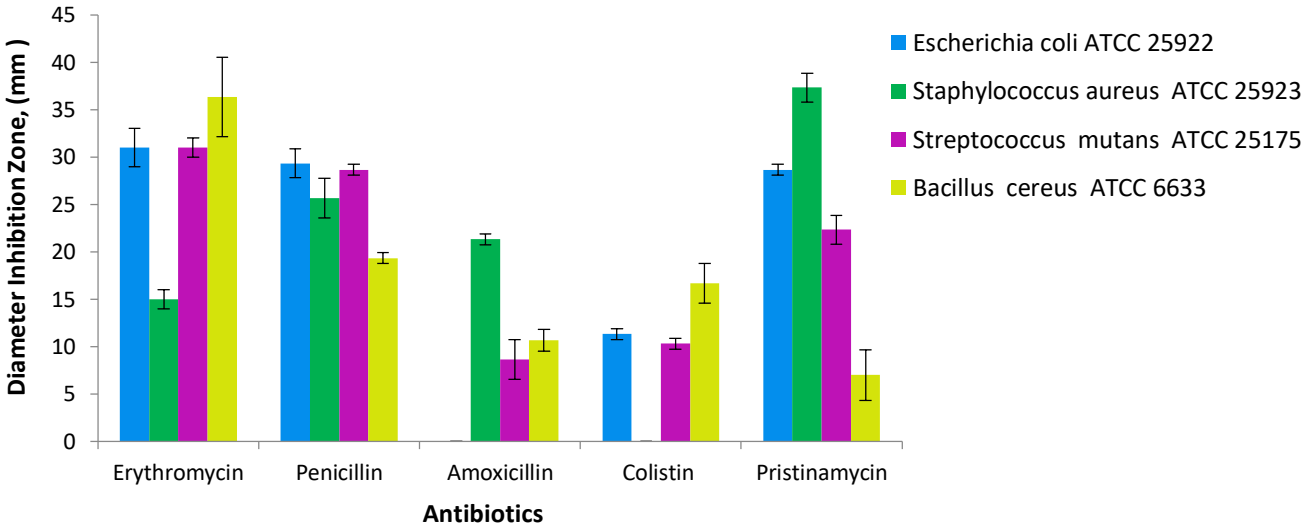

Figure 3. Diameter of Inhibition Zone of Antibiotics Against Selected Bacteria.

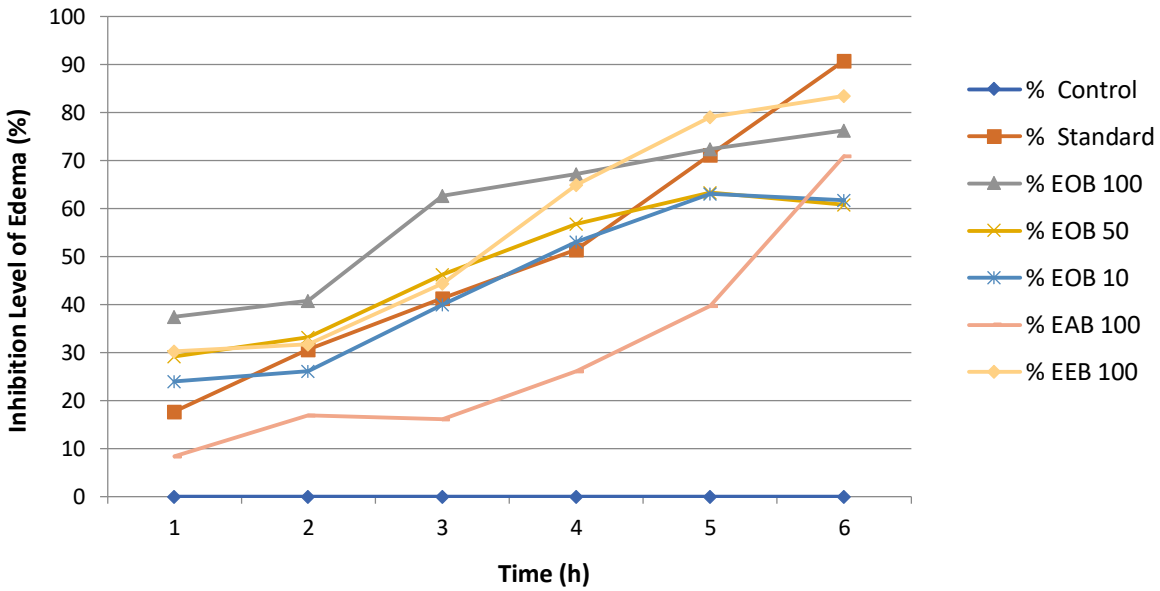

Figure 4. Inhibition Level (%) of Paw Edema by *Ocimum basilicum* Essential Oil, Ethanolic Extract, Aqueous Extract, and Diclofenac During 6 Hours after Carrageenan Injection. Control: Vehicle (NaCl 0.9%); Standard: Diclofenac 10 mg/kg; EOB: Essential oil of *O. basilicum* (100 mg/kg, 50 mg/kg, and 10 mg/kg); EEB: Ethanolic extract of *O. basilicum* (100 mg/kg); AEB: Aqueous extract of *O. basilicum* (100 mg/kg).

(31 ± 2 mm), Penicillin (28.66 ± 0.57 mm), and Pristinamycin (29.33 ± 1.52 mm). In contrast, *S. aureus* is sensitive to Colistin (21.33 ± 0.57 mm), Penicillin (25.66 ± 2.08 mm), and Pristinamycin (37.33 ± 1.52 mm) and resists Amoxicillin. *E. coli*, *S. mutans*, and *B. cereus* are sensitive to E with a diameter ≥31 mm, and are resistant to Colistin antibiotic <7 mm.

Antibacterial Activity Assay and Serial Microdilution Assay

The antimicrobial activity of all plant extracts was evaluated against four pathogenic bacterial strains and one fungal strain. The antimicrobial potential of herbal plant extracts and oil was assessed in terms of the inhibition zone of the microbe's growth (Table 6). Results show that EOB is more

Table 6. The Diameter of the Inhibition Zone of Essential Oil and Basil Extracts Against Bacteria and Yeast

Strains	DIZ (mm)		
	EOB	AEB	EEB
Escherichia coli ATCC 25922	10.16±0.76	13±1	15.16±1.15
Staphylococcus aureus ATCC 25923	15±1	18±1	12.5±0.86
Streptococcus mutans ATCC 25175	13.33±1.04	12.83±0.76	12.16±0.76
Bacillus cereus ATCC 6633	18.16±1.04	15.16±1.04	11.5±0.5
Candida albicans ATCC 10231	15±1	10.83±0.76	11.16±0.28

DIZ: Diameter of Inhibition Zone

Table 7. The Minimal Inhibitory Concentrations (MICs) and (MBCs) of Oil and Extracts Against Bacteria and Yeast

Strains	MIC µl/ml			MBC µl/ ml			MBC/MIC ratio		
	EOB	AEB	EEB	EOB	AEB	EEB	EOB	AEB	EEB
Escherichia coli ATCC 25922	12.5	50	12.5	100	100	25	8	2	2
Staphylococcus aureus ATCC 25923	50	100	25	50	100	25	1	1	1
Streptococcus mutans ATCC 25175	6.25	25	50	50	25	50	8	1	1
Bacillus cereus ATCC 6633	100	12.5	50	200	50	50	2	4	1
Candida albicans 1023	100	50	50	200	100	100	2	2	2

MIC: Minimal inhibitory concentrations MBC: Minimal bactericidal concentrations. MBC/MIC ratio≤2: bactericidal effect. MBC/MIC ratio≥4: bacteriostatic effect.

Table 8. Anti-inflammatory of EOB, AEB, and EEB on Paw Edema Induced by Carrageenan in Mice at a Dose of 100 mg/kg

	Paw Thickness, (mm*)						
	h0	h1	h2	h3	h4	h5	h6
Control	1.91±0.04	2.07±009	3.24±0.24	3.37±0.25	3.57±0.06	3.89±0.11	3.88±0.07
Standard	1.92±0.02	2.05±0.08	2.85±0.10[a]	2.78±0.15[c]	2.73±0.17[c]	2.49±0.08[c]	2.10±0.06[c]
EOB 100	1.87±0.05	1.97±0.14	2.66±0.28[c]	2.42±0.09[c]	2.42±0.25[c]	2.42±0.30[c]	2.34±0.34[c]
EOB 50	1.88±0.04	2.00±0.07	2.78±0.27[b]	2.67±0.21[c]	2.60±0.18[c]	2.61±0.09[c]	2.66±0.19[c]
EOB 10	1.89±0.06	2.01±0.08	2.88±0.14[a]	2.77±0.13[c]	2.67±0.15[c]	2.62±0.14[c]	2.64±0.14[c]
AEB 100	1.91±0.07	2.06±0.06	3.02±0.26	3.14±0.08[a]	3.14±0.10[a]	3.11±0.26[c]	2.48±0.36[c]
EEB 100	1.93±0.02	2.08±0.11	3.11±0.56	3.20±0.17	3.16±0.18[a]	2.86±0.29[c]	2.71±0.41[c]

*Mean ± SEM (n = 6); EOB: Essential oil of *O. basilicum*; Control: vehicle (NaCl 0.9%); Standard: Diclofenac 10 mg/kg. h: hour; a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ significance (comparison with control group); EOB: essential oil of *O. basilicum* (100 mg/kg, 50 mg/kg and 10 mg/kg); EEB: Ethanolic extract of *O. basilicum* (100 mg/kg); AEB: Aqueous extract of *O. basilicum* (100 mg/kg).

active against microbes in comparison with extracts where the EOB inhibits (positive Gram) *S. aureus* ATCC 25923, *B. cereus* ATCC 6633, and *C. albicans* ATCC 10231 with DIZ equal to 15 ± 1, 18.15 ± 1.04, and 15 ± 1 mm, respectively (with MIC ranged from 50 to 100 µl/ml with bacteriostatic effect). The AEB also inhibits (negative Gram): *S. aureus* ATCC 2592318±1 mm with MIC equal to 100 µl/ml and bactericidal effect. *B. Cereus* ATCC 6633 is sensitive to AEB with DIZ equal to 15.16 ± 1.04 mm with MIC of 12.5 µl/ml, while the EEB inhibits *E. coli* ATCC 25922 with DIZ of 15.16 ± 1.15 mm and MIC of 12.5 µl/ml with bactericide effect (Table 7) when compared with the antimicrobial activity of standard antibiotics commonly in use. The EEB is the best inhibitor of *E. coli* ATCC 25922 (15.16 ± 1.15 mm) than CT (10 U) and EOB. AEB is the best inhibitor of *S. aureus* ATCC 25923 than AM (10 U).

Acute Toxicity

Amid analyzing the acute toxicity of the plant, mortality was observed in all mice 24 h after testing EOB at a dose of 600 mg/kg and 1000 mg/kg. A similar observation was noted for AEB at a 1600 mg/kg dose. Sedation and dyspnea were also noted during this dose's first hour after treatment. No mortality was recorded in mice tested at 1000 to 5000 mg/kg doses. The LD_{50} is 470 mg/kg, 400 mg/kg and superior of 5000 mg/kg for EOB, AEB, and EEB, respectively.

Anti-inflammatory Activity

Table 8 indicates the anti-inflammatory activity of EOB, AEB, and EEB against carrageenan-induced paw thickness. Figure 4 presents the edema inhibition. In the present study, the EOB (100 mg/kg) offers the best inhibition (75.35%) of edema after 6 h of induced Carrageenan at a rate of 3 h at 50 mg/kg and 10 mg/kg compared with the control with inhibition at 59.40% and 58.48% of edema respectively. The AEB and EEB extracts inhibit 71.00% and 60.43% of edema, respectively. All inhibitions are less than the standard (10 mg/kg), which inhibited 90.11% of edema paw. The EOB (100 g/kg) provides a good inhibition of edema better than that of Diclofenac (10 mg/kg) from the second hour (2.66 ± 0.28 mm and 2.85 ± 0.10 mm, respectively) to the fifth hour (2.42 ± 0.30 mm and 2.49 ± 0.08 mm, respectively) after induced of Carrageenan, with a highly significant decrease of edema ($p<0.001$). This result indicates that it has a faster effect. It is also interesting to note that Diclofenac has a good effect in the sixth hour.

Dose Conversion (Mice to Humans)

EO toxicity and the *O. basilicum* extracts on mice were experimentally investigated, and LD_{50} was identified. The dose was thus transferred from animal to human using the K_m factor. The converted dose per kg body weight of LD_{50} of EOB (470 mg/kg) on mice was 38.11 mg/kg for humans.

Table 9. Human Equivalent Dose Calculation of Experiment Dose of LD_{50} Toxicity Using Body Surface Area as Scaling Unit

Extracts	LD50 dose mg/kg	Converted dose per kg BW	Converted dose according to human BW examples		
			70 kg	60 kg	50 kg
EOB	470	38.11 mg/kg	2667.70 mg	2286.60 mg	1905.92 mg
AEB	400	32.44 mg/kg	2270.88 mg	1946.4 mg	1622.00 mg
EEB	5000	405.515 mg/kg	28386.05mg	24330.6 mg	20275.5 mg

K_m Value Mouse = 3.00, K_m Value Human = 37.00. Formula: Human Equivalent Dose (mg/kg) = Dose to be converted/(Human K_m/Mouse K_m).

Table 10. Human Equivalent Dose Calculation of Experiment Dose of Anti-inflammatory Using Body Surface Area as Scaling Unit

Extracts	Experience Does mg/kg	Converted Dose Per kg BW	Converted Dose According to the Human BW Examples		
			70 kg	60 kg	50 kg
AEB	100	8.11 mg/kg	567.57 mg	486.49 mg	405.41 mg
EEB	100	8.11 mg/kg	567.57 mg	486.49 mg	405.41 mg
EOB	100	8.11 mg/kg	567.57 mg	486.49 mg	405.41 mg
EOB	50	4.05 mg/kg	283.78 mg	243.24 mg	202.7 mg
EOB	10	0.81 mg/kg	56.76 mg	48.65 mg	40.54 mg

K_m Value Mouse = 3.00, K_m Value Human = 37.00. Formula: Human Equivalent Dose (mg/kg) = Dose to be converted/(Human K_m/Mouse K_m).

The converted dose of the LD_{50} of AEB (400 mg/kg) equals 32.44 mg/kg. The converted dose of LD_{50} of EEB (5000 mg/kg) is very high (405.51 mg/kg) because this extract is not toxic (Table 9). The converted dose per kg body weight of experimental anti-inflammatory (100 mg/kg) in mice equals 8.11mg/kg in humans. The converted dose of (50 mg/kg) was 4.05 mg/kg and the dose (10 mg/kg) is 0.81 mg/kg (Table 10).

Discussion

Benabdallah et al. (2020)[24] reported various uses of *O. basilicum* L. in eastern Algeria. This plant is used as digestive (12.39%), stomachic, sedative (7.08%) agents, and as a spice (6.19%). In contrast, decoction (30.56%) and infusion (23.61%) are the most common methods of preparation of fresh or dry leaves (15.28%). The recipe in the ethnobotanical study is in accordance with the study carried out by Ahmadifard et al. (2020)[25] regarding the impact of basil EO on controlling migraine attacks when the compound is taken orally or through inhalation. Seeds of *O. basilicum* L. are used in traditional medicine for stomach ulcers, dyspepsia, diarrhea, and pharyngitis.[26]

O. basilicum has also been reported as a promising and powerful plant in other regions worldwide. For instance, *O. basilicum* is used in Turkey to prevent and treat diabetics and cardiovascular disorders. In India, Siddha medicine uses *O. basilicum* to treat pimples on the face. Traditionally basil has been used to treat headaches, coughs, diarrhea, and kidney malfunctions. It is worth mentioning that it is also used in treating insect stings, snake bites, and external skin infections. In Bulgaria, it is used as a folk medicine to treat aches and pain. In Spain, it is used as a sedative and for kidney inflammation.[27] Hot tea of basil plant leaves treats nausea, dysentery, and intestinal gas. Similarly, basil has been used to treat critical health conditions, including cancer, convulsion, deafness, epilepsy, insanity, sore throat, toothaches, and whooping cough. Alternatively, *O. basilicum* is a source of EOs in industries, perfumery, oral products, and traditional ritual.[28]

The yield can be explained by the ability of decoction to extract the maximum of compounds compared with ethanol. Goudoum (2017)[29] obtained an oil yield of 2.32%. In contrast, Hamed et al. (2017)[30] obtained a yield of 0.7%. EOB is a yellowish or greenish-colored liquid. Benabdallah et al. (2020)[24] reported extracts yield between 15.24% and 28.67%. The yield of EO depends on the cutting time, while the quality of basil depends on a variety of basil's growing technology and environmental factors such as temperature, rainfall, photoperiod, relative humidity, and irradiance.[31] Nonetheless, irrigation, especially via sprinklers, may cause some dangerous diseases and contribute to a decrease in the content of the EO.[32]

The EOB species extracted from the aerial part by hydrodistillation containing major constituents are linalool (40.5 to 48.2%) and methyl chavicol (estragole) (28.9 to 31.6%).[33] Among European basil cultivars, the most valuable are those rich in linalool and methyl chavicol.[32] Zhang et al. (2009) identified linalool (about 30.0%) and (Z)-cinnamic acid methyl ester as the main components, followed by cyclohexene in the EO of the aerial parts of *O. basilicum* L.*var. pilosum*.[34] Chemical analysis of the EO from *O. basilicum* cultivated in the Algerian Saharan Atlas revealed 26 unique compounds, with linalool (52.1%) and linalyl acetate (19.1%) as the major compounds.[35]

The results of this study indicate that *O. basilicum* contains about 20 compounds, such as linalool, estragole, methyl eugenol, 1, and 8-cineole: they were identified by GC-MS.[27] Slougui et al. (2015)[36] reported two main compounds for the Mostaganem basil: linalool (50.5%) and geranial-neral (21.5-17.5%), respectively. Brada et al. (2011)[37] indicated that the oil of *O. basilicum* contains linalool (44.7%) and linalyl acetate (14.0%). The analyses in south Tunisia basil showed that major compounds of EOB are linalool (29.23%), methyl cinnamate (18.97%), and eugenol (5.84%), followed by 1,8-cineole (5.74%).[38]

Plant Benzoic acids and their derivatives are common and widespread mediators of plant responses to biotic and abiotic stress.[39,40] Biosynthesis of all plant BAs and their products

ultimately starts from the shikimate pathway.[41] The Benzoic acid is converted to Salicylic acid in plants.[42] Many natural products derived from plant BAs or benzoyl/benzyl moieties are also promising medicinal or nutritional substances for humans.[41] Kwee and Niemeyer (2011)[43] reported that the major phenolic compounds of *O. basilicum* are rosmarinic, chicoric, and caffeic acids. In addition, rutin, quercetin, and ferulic acid have been reported as other phenolic compounds. Kim et al. (2006)[44] obtained two main phenolic compounds, rosmarinic acid, and caffeic acid. The Romanian *O. basilicum* extract is rich in ferulic acid, presenting a 437.58 mg/100 g extract.[45]

The results clearly indicate that EOB has an antioxidant activity with 88.42 % inhibition of DPPH in 2 mg/ml. These results are not consistent with those reported by Hadj Khelifa et al. (2012)[46] for basil in northern Algeria. The concentration of the EOB needed to scavenge 50% of DPPH is 83.54 mg/ml. Our result is less than the result obtained by Hussain et al. (2008)[47] for the EO from the aerial parts of Pakistani basil. Their findings exhibited good antioxidant activity as measured by DPPH with IC_{50} between (4.8 µg/ml) and (6.7 µg/ml) depending on the harvest season. The EEB showed moderate scavenging effects against radical DPPH compared with the study of Ghasemzadeh et al. (2016)[48], who concluded that the IC_{50} value of basil extract was 78 µg/ml, whereas ascorbic acid has an IC_{50} of 41 µg/ml.

Basil is a natural source of antioxidants that can neutralize free radicals, which are harmful to human health,[49] as the high antioxidant value of herbs is appropriate for the presence of organic acids, vitamin C, provitamin A, phenolic compounds, and anthocyanins. The oils with higher phenolic content exhibit higher radical scavenging abilities.[2] The Egyptian basil extracts contained notable levels of total phenolic contents and exhibited good DPPH radical scavenging capacity which was reported to be higher than that of Eos.[50] In contrast, in this study, the antioxidant properties were correlated with the highest linalool (terpene alcohol) content. *O. basilicum* displays great potential for antibacterial activity against *Bacillus cereus*, *B. subtilis*, *S. aureus*, and *E. coli*, with their respective zones of inhibition of 11.2-21.1 mm and MIC values of 62.5-500 µg/ml.[51] The EO of basil shows mild inhibition against *E. coli* and *B. cereus.*[52] Similar results were demonstrated by Amor et al. (2021) on antimicrobial activity. The study manifested a significant inhibitory effect against all the microorganisms tested: *E. coli*, *Staphylococcus*, and *Streptococcus*, mostly Gram-positive bacteria[53] but less than the standard antibiotics.[54]

Moreover, the results of antimicrobial assays reported by Hussain et al. (2008)[47] indicated that linalool is the most abundant component against bacterial strains: *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*. The authors reported that all the tested microorganisms were affected. The findings of this study suggest that the EO of *O. basilicum* may have potential use in the food and/or pharmaceutical industries as antimicrobial agents.

The oil of basil is moderately toxic based on the scales of toxicity set by Hodge and Sterner. Venâncio et al. (2011)[20] indicated that there are no signs of toxicity or mortality under 250 mg/kg. The LD_{50} was 532 mg/kg and may be related to the higher linalool content because it's reported intraperitoneal LD_{50} is well documented in the literature ranging from 200 to 1200 mg/kg. In another study conducted by Chaudhary et al. (2016),[55] the LD_{50}findings suggest that the animals are safe up to a maximum dose of 2000 mg/kg body weight. The biological evaluation was carried out at 100 mg/kg body weight doses. The significant anti-inflammatory activity of all extracts and the standard drug observed in the present study can be correlated to the inhibition of mediators of inflammation such as Histamine, Serotonin, and Prostaglandin. The results of the anti-inflammatory activity of EOB are mainly attributed to its main compound, Linalool inhibits inducible nitric oxide synthase and thus the pro-inflammatory effects of NO, which causes vasodilation.[56] Linalool and Linalyl acetate show anti-inflammatory activity on the edema of paw-induced mouse carrageenan.[3] Carrageenan-induced paw edema is usually utilized in experimental model of acute inflammation represented by a biphasic development of edema. The first phase (1-2 h) is dependent on mediator release, e.g., Histamine, Serotonin, and Bradykinin.[57] At the same time, the second phase (3-6 h) is sustained by the release of Prostaglandins, Leukotrienes, Lysozymes, Proteases, NO, and by local infiltration by neutrophils producing free oxygen radicals, e.g., O^{2-} and OH.[58]

Extrapolating doses from one species to another solely based on body weight may not be the method. This is due to the fact that, pharmacokinetic activity and physiology are also prominent in suboptimal as the dose may lead to adverse effects when exploring experimental therapies on humans.[23] Key factors to consider when scaling the dose are that larger animals have a lower metabolic rate, slower physiological processes, and require a lower drug dose on a weight basis.[59]

Conclusion

Natural bioactive compounds are substantial in managing global health issues. This study presented the ethnobotanical, biological activities, and chemical composition of *Ocimum basilicum.* L *var Genovese*. The results show that the tested basil in western Algeria can be classified as a linalool chemotype. It can be used as aroma additives in food, pharmaceuticals, and cosmetics and is a source of antioxidants. *Ocimum basilicum* has an antimicrobial effect and is a good anti-inflammatory. The novel approach of our study confirms the ethnobotanical study and its uses in traditional medicine in Algeria. In general, the EO showed stronger biological activity than the extracts. The aqueous extract of Algerian

Ocimum basilicum could be a potential source of benzoic acid. Furthermore, the results provide new perspectives for future analyses on the synthesis of food preservation and salicylic acid from this species in Algeria.

Authors' Contributions

FFF practiced in the laboratory experiments and wrote the article. MB, ML, and AT developed the research plan and proofread the article. MY performed the antimicrobial activity. BN, PA, and FD analyzed and interpreted the GC/SM. YSK analyzed and interpreted the HPLC of extracts.

Ethics Approval

This study protocol was approved by the Local Ethical Comity of the University, based on adequately performed laboratory and animal experimentation according to the Helsinki Declaration (1964).

Conflict of Interest Disclosures

The authors declare that they have no conflicts interest.

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