International Journal of Medicinal Plants and Natural Products (IJMPNP)
Volume 7, Issue 4, 2021, PP 1-11
ISSN 2454-7999(Online)
DOI: https://doi.org/10.20431/2454-7999.0704001
www.arcjournals.org

Antioxidant, antimicrobial and anti-inflammatory activities of ethanol extracts and essential oil from the Tunisian *glycophyte Ocimum basilicum* L.

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Abstract: *Medicinal plants constitute noteworthy sources of phytochemical metabolites with potential therapeutic effects. In this work, ethanol (EtOH) extract with decreasing polarity and essential (EO) were screened for antioxidant, antibacterial, antifungal and anti-inflammatory properties from the leaves of Basil (Ocimum basilicum L.). In addition, chemical characterization of their bioactive molecules was performed using HPLC system, the essential oil was also analyzed by GC/MS. The results indicated that EtOH 70% and EOs were the most promising regarding antioxidant (and antimicrobial abilities, possibly due to its richness in valuable phytochemical compounds. In the same tendency, EOs and EtOH 70% showed a noteworthy anti-inflammatory activity inhibiting NO release with an IC50 values equal to 20 and 26 µg/mL. In our study, we concluded that EtOH 70% (green extracts) and EO can be used as potent natural antioxidants and antifungal agent beneficial for human health.*

Keywords: *Ocimum basilicum, ethanol, essential oil, biological activities*

1. INTRODUCTION

Although World Health Organization (WHO) reported that the principal health care system for the 60% population of the world is represented by the folk medicines. Yet a great number of plant species with potential biological activities were unexplored [1]. Besides, the potency of traditional medicines is now a putative fact owing to their compatibility with human body and lesser side effects [2].

Medicinal plants represents inexhaustible source of natural antioxidants that may protect human body systems against harmful effects of oxidative stress. The use of plants as source of antioxidants to enhance health and food preservation is one of our current interest and it is well known that there is positive associations between the assumption of diets rich in fruits and vegetables and the prevention of diseases [3].

On the other hand, synthetic antioxidants are widely used in food industry; however, their utilization has been interrogated due to their toxicity. Thus, there is an escalating interest to the use of natural antioxidants in order to reduce or restore synthetic antioxidants [3]. Bioactive molecules (biomolecules) are known by their aptitude to delay or inhibit cellular damage owing to their property to quench free radicals [3], and to remove ROS/RNS incitors [4].

Phenolic compounds constitute the major group of natural antioxidant molecules; that possess a potential properties in neutralizing free radicals and protecting cells against their harmful effects. Furthermore, they are characterized by their potent antioxidant, antibacterial, anti-inflammatory ,anti-diabetic antispasmodic, immunomodulating, antioedematogenic, analgesic activities [5].

Likewise, essential oil (EO) represents a valuable extracts of aromatic and medicinal plants due their potential biological activities (antibacterial; antifungal; antioxidant properties…..).

The genus *Ocimum*. a member of the Lamiaceae family. contains more than 150 species of herbs and shrubs [6]. Several species from Lamiaceae family are used in many enthnomedicinal vertues. Basil

(*Ocimum basilicum* L.) is a priceless medicinal and aromatic plant chiefly due to its EO extracted from leaves and fowers used in food as a favoring agent [6]. Besides, it has a characteristic odor and sharp taste issue from its secondary metabolites including polyphenols [6].

Basil is also considered a source of aromatic compounds and essential oils containing biologically active constituents that possess insect repellent, antibacterial, antifungal and antioxidant activities [7]. Basil is economically important due to the use of its essential oils being used in hygiene and cleaning products, perfumes, cosmetics and local anesthetic and antiseptics [8].

Substitution of organic solvents with an environmentally favorable solvent for the extraction of biomolecules is indeed of importance in terms of green chemistry and sustainability. Indeed, ethanol is classified as an environmentally preferable green solvent as it is produced by fermenting renewable sources. In comparison with other solvents, ethanol is a relatively low-cost solvent and readily available [9].

This work was conducted to explore for the first time the antioxidant , antimicrobial and anti-inflammatory activities of eluted ethanol-water mixtures of decreasing polarity (EtOH 5%, EtOH 30%, EtOH 70% and EtOH 100%) and essential oil of *Ocimum basilicum* grown in Tunisia with consideration to their chemical compositions in order to explore the ecological solvants (non toxic) in biological activiities .

2. MATERIALS AND METHODS

2.1. Plant Sampling and Obtention of Phenolic-Rich Extracts

Leaves of *Ocimum basilicum* L was collected in April 2021 from plants cultivated in Biotechnology center of the Technopark of Borj Cedria (36°51’59’’ North 10°9’53’’East. Governorate of Nabeul in North of Tunisia). The harvested plants were identified by Pr Abderrazek SMAOUI (Botanist). Voucher specimens [HPM37] was deposited in the herbarium of the Laboratory of of Aromatic and Medicinal Plants (LPAM). The phenolic rich-extract (PRE) was prepared by adding 100 mL of eluted ethanol-water mixtures of decreasing polarity (EtOH 100%. EtOH 70%. EtOH 30% and EtOH 5%) to 10 g of

basil powder. After ultrasonic maceration for 30 min, extract was filtered, evaporated and finally lyophilized. The dry residue is weighed and stored at 4°C for subsequent analysis.

2.2. Quantification of Phenolic Compounds

The total phenolic content (TPC) was assessed using a colorimetric assay based on the Folin-Ciocalteu reagent. A volume of 125 µL of BE (basilica extract) was added to 60 µL of H_2O and 15 µL of the Folin-Ciocalteu reagent (0.2 mol. L^{-1}). After shaking 150 µL of Na_2CO_3 (7%) was added. After incubation for 1 hour at room temperature the optical density (OD) at 750 nm was determined. Results were expressed as mg of gallic acid equivalent per g of dry weight (GAE g^{-1} DW) using a calibration curve [10]. For total flavonoid content (TFC). 250 µL of BE was mixed with 75 µL $NaNO_2$ (5%; w/v). Then, 150 µL of $AlCl_3/6H_2O$ (10%; w/v) and 500 µL of NaOH (1 M) were added after 6 min of incubation. After adjusting the volume to 2500 µL with H_2O. the absorbance was determined at 510 nm. TFC were expressed as mg (+)-catechin equivalent/g DW (mg CE. g^{-1} DW) [10].

Proanthocyanidin were revealed according to vanillin-H_2SO_4 method. Fifty µL of BE from each treatment were pipetted out into a test tube. Then, 3 mL of 4% methanolic-vanillin solution and 1.5 mL of concentrated H_2SO_4 were added and vortexed. Tubes were stand for 15 min. The absorbance was measured at 500 nm. The amount of condensed tannins were expressed as mg catechin equivalent/ g DW (mg CE. g^{-1} DW) [11].

2.3. Apparatus and Chromatographic Conditions for the Analysis of Polyphenols

The identification of compounds was done using HPLC system equipped with a reversed phase C_{18} analytical column of 4.6 x 100 mm and 3.5 µm particle size (Zorbax Eclipse XDB C_{18}). The DAD detector was set to a scanning range of 425 nm. Temperature of column was maintained at 25°C. The volume of injected extract was 2 µL and 0.4 mL/min was the mobile phase flow-rate. Mobile phase B was milli-Q water constituted of 0.1% formic acid and mobile phase A was methanol. The optimized

chromatographic condition was as follows: 40% A and 60% B. Phenolic compounds identification were obtained by comparing their retention time and the UV spectra with those of pure standards. The solvent gradient consisted of a series of linear gradients. starting with 15–28% of solvent B over 5.6 min. increasing to 29% at 8.8 min. 100% of solvent B at 13.1 min and keeping up to 17 min. followed by the return to the initial conditions with total running of 20 min [11].

For quantitative analysis, the limits of detection and quantification were calculated from the parameters of the calibration curves obtained by injection of known concentrations of different standard compounds namely ferulic acid (y = 20.505x -8.728; R^2 = 1.000). rosmarinic acid (y = 8, 494x+ 71.265; R2 = 1.000), isorhamentin (y = 22.34 x-1.56; R^2 = 0.99) and quercetin (y = 9.58 x - 7.41; R^2 = 1.00) [11].

2.4. EOs Isolation

Samples collected were air dried in the shadow at room temperature then partially smashed manually before extraction. Triplicate samples of 100 g were subjected to hydrodistillation in 1L of deionized water using a Clevenger apparatus for up to 6 h, time which was necessary for a complete extraction. The oils obtained were dried over anhydrous sodium sulphate, filtrated and storedat +4°C until tested. EOs yield was expressed as percent of the plant material used.

2.5. Gas Chromatography (GC) Analysis

GC analysis was carried out according to Chimnoi et al. (2018) [12], using an Agilent 6980 gas chromatograph equipped with a flame ionisation detector and split-splitless injector attached to HP-INNOWAX polyethylene glycol capillary column(30 m 0.25 mm). One micro-liter of the sample (dissolved in hexane as 1/50 v/v) was injected into the system. The constituents were identified by comparing their relative retention times with those of authentic compounds injected in the same conditions.

2.6. In *Vitro* Evaluation of Antioxidant Capacities from Eluted Ethanolic Extracts and Essential Oil

Total antioxidant capacity (TAC) was assessed according to Saada et al. (2014) [13]. Briefly. 100 µL of diluted BE was combined to 1 mL of reagent solution (0.6 M sulfuric acid. 28 mM sodium phosphate and 4 mM ammonium molybdate). The mixture was allowed to cool. after incubation at 95°C for 90 min. The absorbance was measured at 695 nm and TAC was expressed as mg gallic acid equivalent / g DW (mg GAE. g^{-1} DW).

Antiradical capacity of BE against 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical was assessed according to Saada et al. (2014) [13] method. Briefly, 250 µL of ethanolic solution of stable radical DPPH (0.2 mM) was added to 1000 µL of increasing concentrations of BE. After 30 min of incubation at room temperature. the absorbance was read against a blank at 517 nm. DPPH scavenging ability was expressed as IC_{50} (mg mL^{-1}) which is the inhibiting concentration of 50% of the synthetic radical. The inhibition percentage (IP %) of DPPH radical was calculated using the following formula:

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

In the same trend antiradical ABTS scavenging ability was expressed as IC_{50} (mg/mL) which is the inhibiting concentration of 50% of the synthetic radical.

Ferric reducing antioxidant power (FRAP) was focused on the reduction of the trivalent iron produced by the $FeCl_3$ [10]. The intensity of the blue-green color was measured at 700 nm. Values were expressed as EC_{50} (mg/mL): the effective concentration of BE corresponding to an OD = 0.5.

For β-Carotene bleaching test (BCBT), 200 µg of β-carotene was dissolved in 20 mL chloroform. A total of 4 mL of this solution was then mixed with 40 mg linoleic acid and 400 mg Tween 40. Chloroform was evaporated under vacuum at 40°C and 100 mL of oxygenated ultra-pure water was added. Then the emulsion was vigorously shaken. An aliquot (150µL) ofthe β-carotene: linoleic acid emulsion was distributed in the 96wells of the microtitre plates and solutions of the test samples (10µL) were added. Three replicates were prepared for each ofthe samples. The microtitre plates were incubated at 50°C for120 min. and the absorbance was measured using a model EAR400 microtitre reader

(Labsystems Multiskan MS) at 470 nm. Readings of all samples were performed immediately (t =0min) and after 120 min of incubation. The antioxidant activity of the extracts was evaluated in term of β-carotene blanching using the following formula:

β-carotene bleaching inhibition (%) = [S-C_{120}] / C_0-C_{120}*100

where $C_0$and $C_{120}$are the absorbance values of the control at 0 and120 min respectively and S is the sample absorbance at 120 min. The results were expressed as IC_{50}values (µg/mL) [13].

2.7. Antimicrobial Activity from Eluted Ethanolic Extracts and Essential Oil

The antimicrobial activity of BE was assessed by the agar disk diffusion assay against three *Candida* species (*albicans* ATCC 90028. *glabrata* ATCC 90030 and *krusei* ATCC 6258) and six human pathogenic bacteria: Gram-negative bacteria including *Echerichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 4352and Gram-positive bacteria including *Staphylococcus aureus*ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Micrococcus luteus* ATCC 4698. Bacterial yeast and fungal spores suspensions were standardized at 10^8 CFU/mL (0.5 of Mac Farland). 10^7 CFU/mL and 10^6 spore/mL for fungi. respectively. Suspensions were spread on Muller-Hinton Agar (MHA) and Potato Dextrose Agar (PDA) [13].

2.8. NO˙ Production from Eluted Ethanolic Extracts and Essential Oil

RAW 264.7 cells were seeded in 24- well plates at 5×10^4 cells/well. After 24 h of incubation cells were pretreated for one hour with different concentrations of BE and EOs before 24h-stimulation with 1µg.mL^{-1} of lipopolysaccharide (LPS). Griess reagent (1% sulfanilamide (Sulf). 5% phosphoric acid and 0.1% N-1-napthylethylenediamine dihydrochloride) was used to determine the accumulation of nitrite in culture supernatant. The test was performed by mixing 100 µL of cells' supernatant with the same volume of Griess solution. The resazurin test was performed to check the cytotoxicity of samples on cells. N(G)- nitro-L-arginine methyl ester (L-NAME) was used as a positive control. The absorbance was then determined at 540 nm and the content of nitrite was calculated referring to $NaNO_2$ standard curve [11].

2.9. Statistical Analyses

Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test was performed. The statistical tests were applied using Graph Pad Prism version 6 and the significance level was $p < 0.05$. The Pearson's correlation was done using XLSTAT considering variables centered on their means**.**

3. RESULTS AND DISCUSSION

3.1. Effects of Solvent Polarity on Phenolic Pools

Ethanol (EtOH) is known to be an efficient solvent for phenolic compounds extraction owing to its safe human consumption. Results showed that *O. basilicum* phenolic contents varied considerably as function of solvent polarity. Indeed, ethanol/water (70/30.v/v) extract produced the highest total polyphenol amounts (67 mg GAE/g DR) closely followed by pure EtOH (61 mg GAE/g DR). Contrawise, EtOH 30% and EtOH 5% represented the less amounts of polyphenols (41 and 40 mg GAE/g DR respectively).

In the same tendency TFC extracted with ethanol/water (70/30, v/v) displayed the uppermost contents (90.48 mg CE/g DR) followed by pure ethanol, then EtOH 30% (96.17 mg CE/g DR) and EtOH 5% (68.06 mg CE/g DR). As well, condensed tannins were more produced in EtOH 70% (19.83 mg CE/g DR) than other extracts.

These results were in agreement with previous studies which showed that solvent nature exert a great power in phenolic extraction capacities [14].

In a study conducted by Veronezi et al. (2006) [15] using ethanol for extraction, TPC were 83.74 mg GAE/g for basil extract. Juliani and Simon (2002) [16] reported that green basilica cultivars evaluated yielded significantly total phenols varying from 35.6 mg in 'Cinnamon' to 62.9 mg in 'Italian Large Leaf'. It has been observed that the phenolic compound extraction is proportional to the solvent polarity.

In fact, the higher polarity of solvent extraction produce the greater amount of extracted phenolic compounds [16].

Besides the addition of 30% distilled water to the solvent extract can influence considerably the extraction capacity of phenolics in *O.basilicum*. In fact. extracting phenolics by aqueous mixtures of ethanol and acetone were respectively superior by 1.4- and 1.5-folds as compared to the same pure solvents in the extraction capacity of plant polyphenols (Trabelsi et al. 2010) [17]. In this way, Chavan et al. (2001) [18] reported that EthOH 70% was more efficient than absolute ethanol for recovery of maximum condensed tannins amount from different pea cultivars. These results demonstrate clearly the influence of the solvent polarity on the extractability of phytochemical compounds. As for our results, Wang et al. (2013) [19] showed that water addition to the solvent improved the flavonoids extraction especially for ethanol. methanol or acetone.

In parallel eluted *O. basilicum* EtOH extracts were analyzed by HPLC–DAD at 280 nm. Peaks were identified by comparing their retention time and UV spectra with chromatograms using reference standards in the same conditions. The retention times (Rt) are listed in Table 1. We obtained good separation for most peaks. The HPLC–DAD analysis allowed identifying peaks corresponding to 2 phenolic acids (ferulic acid and rosmarinic acid) and 2 flavonoids (isorhamentin-3-rutoniside and quercetin).

Out of all phenolics, isorhamentin was the represented metabolite in all extracts with a values varing from 11.45 to 6.02 mg/g DR. Rosmarinic acid was the second most abundant phenolic compound in basil with higher values were recorded in EtOH 70% extract (7.89 mg/g DR). Ferulic acid, the third predominant component in all extracts, which EtOH 70% depicted the superior quantity (5.74 mg/g DR). Although, quercetin was just detected in EtOH 70% extract with low amount (1.21 mg/g DR).

Comparing with the different investigation in the current interest, the discrepancy between studies could be attributed to chemotype, environmental and climatic condition, extraction and quantification method plant populations, genetic (genus, species and ecotype). Thus, it's quite interesting to observe such profil variability in the same country in order to justify the different uses of these plants.

Table1. *Phenolic compounds identified and quantified by HPLC from phenolic rich extract of eluted ethanol-water mixtures from O. basilicum. Means (three replicates) followed by the same letter are not significantly different at P <0.01.*

	TPC (mg GAE/g DR)		TFC (mg CE/g DR)		TCT (mg CE/g DR)		
EtOH 100%	61.74±1.4 ^{B}		71.49±6.3^{B}		40.16± 3.8^{C}		
EtOH 70%	67.02±3.8^{A}		90.48± 11.2^{A}		71.00± 10.9^{A}		
EtOH 30%	41.05±5.2 ^{C}		69.17± 3.4^{C}		50.16±1.2 ^{B}		
EtOH 5%	40.17±0.6 ^{C}		68.06±7.9 ^{C}		48.50± 4.9^{B}		
Compounds	RT	CE	CC (R^2)	EtOH 100%	EtOH 70%	EtOH 30%	EtOH 5%
Ferulic acid	11.67	20.505x - 8.728	1.00	4.23^{B}	5.74^{A}	1.58^{D}	3.22^{C}
Rosmarinic acid	12.70	y=22.28x+1.6	0.99	5.34^{B}	7.89^{A}	2.78^{D}	4.04^{C}
isorhamnetin 3-*O*-glucoside.	13.82	y = 22.34 x-1.56	0.99	9.95^{B}	11.45^{A}	6.02^{D}	8.34^{C}
Quercetin	16.36	y= y = 9.58 x - 7.41	0.99	-	1.21	-	-

Means followed by the same letter are not significantly different at P ≤0.05

3.2. Chemical Composition of the Essential Oil of *O. Basilicum*

The essential oil of *O. basilicum* was subjected to detailed GC–MS analysis. The yield of oils was 67%. Exactly 30 compounds were identified in this sample (Table 2, Figure 1), representing 89.02% of the total oil. The most abundant components was monoterpene oxygenetaed -group tentatively assigned to linalool (44.52%). Hydrocarbonated monoterpenes compounds were once presented mainly represented by limonene (13.24%) and other compenets with low percentages citing camphene

(0.25%), α-pinene (0.90%), δ-3-carene (0.46%), *p*- cyrnene cymene (0.43%) andγ-terpinene (0.35%). Hydrocarbonated and oxygenated sesquiterpene groups were also present with moderate percentages.

Linalool was the major compound in basil essential oil in Italy [20, 21] and United States [22]. Besides, The presence of methyl chavicol in basil EOs has been reported in genotypes in Yemen, USA, Thailand, UnitedKingdom [23], Brazil [24], and local varieties in Turkey [6].

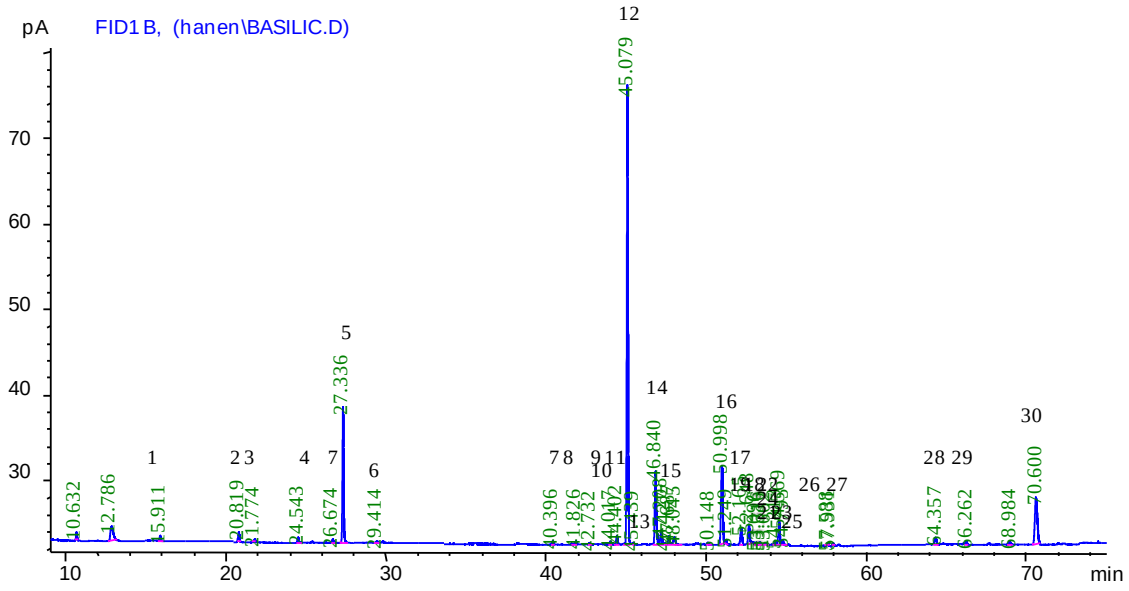

Figure1. *GC/MS profil of O. basilicum.*

A numerous factors, including genetic, geo-climatic zones or growing conditions such as the dose of macro/micro-nutrients, temperature, soil nature, humidity, day length, altitude, and the amount of available water, influence the chemical composition of EO. It also depends on season or phenological stage of plant (i.e. vegetative, flowering and fruiting stages) [25]. Consistent with these factors, plant biosynthetic pathways can alter the relative proportions of the EO components.

Table2. *Essential oil composition (%) of O. basilicum.*

Compound N°	RT (mn)	(%)	Identified compound
1	15,91	0,41	tricylene
2	20,81	0,90	α-Pinene
3	21,77	0,25	Camphene
4	24,54	0,46	Δ- carene
5	27,33	13,24	Limonene
6	29,41	0,18	1,8-cineol
7	40,39	0,35	*y*-Terpinene
8	41,82	0,43	p-Cyrnene
9	42,73	0,19	Terpinolene
12	45,07	44,52	Linalool
14	46,83	7,71	Methyl chavicol
15	47,20	1,97	Caryophyllene oxide
16	50,99	8,80	Estragole
18	52,16	1,89	Terpinene 4 ol
19	52,64	2,22	β caryophylene
22	53,66	0,25	Borneol
23	54,31	0,40	Geraniol
24	54,56	2,37	Geranyl acetate
25	54,79	0,47	α-Humulene
26	57,58	0,25	Germacrene
27	57,93	0,33	δ-Cadinene
28	64,35	0,88	α-Copaene
30	70,60	8,92	Methyl Eugenol

3.3. Effects of Solvent Polarity and Essential Oil on Antioxidant Activities

The antioxidant ability of *O. basilicum* as function of EtOH decreasing polarity and EO was investigated through *in vitro* methods namely DPPH; $ABTS^{\cdot+}$, FRAP and *β*-carotene bleaching in order to evaluate their quenching ability toward DPPH/ABTS radicals, as well as the ability to reduce Fe^{3+} to Fe^{2+} and to hamper the bleaching of the antioxidant pigment β-carotene respectively. Results analysis depicted that EO exhibited the higher IC_{50} values to trap $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ radicals (IC_{50}= 200 and 300 µg/mL repectively) followed by EtOH70% (IC_{50}=420 and 380 µg/mL); while EtOH 5% and EtOH 100% extracts revealed the lowest DPPH and ABTS scavenging activities with an IC_{50} values equal to 560,600, 740 and 620 µg/mL respectively. For RPC (reducing power capacity) EO was once distinguished by the interesting activity with respective EC_{50} values of 540 µg/mL (Table 3). Analysis of *β*-carotene/linoleic acid oxidation was also consistent with data obtained from the anti-radical and Fe-reducing tests and proved that EO of such species has the potent activity since it proved the lowest IC_{50} compared to the EtOH extracts.

The antioxidant efficiency of EOs was reported in many previous works and seems to be related to the activity of some kinds of compounds enclosed in such as oxygenated monoterpenes) [26]. On the other hand the strongest ability of EtOH extract to quench DPPH□ and ABTS□$^{+}$ radicals could due to the omnipresence of phenolics compounds with hydroxyl group attached to the aromatic ring structures [27]. This ability might be also related to the presence of secondary metabolites such as terpenoids, vitamin C and iroids [27].

In contrast a recent study conducted by Molan et al. (2012) [28] reported that water extract of *O. basilicum* had the highest FRAP activity compared to the ethanolic extract. These results suggest that the plant extract might react with free radicals particularly with peroxy radicals which are the major propagators of the auto-oxidation of fat.this is by terminating the chain reaction. The phenolics and other chemical compounds present in the ethanol extract may suppress lipid peroxidation through different chemical mechanisms such as free radicalscavenging. electron transfer radical addition or radical recombination [29].

Table3.*Variation of DPPH,ABTS radicals scavenging. ferric reducing antioxidant power (FRAP) and β-Carotene bleaching test (BCBT) activities of eluted ethanolic extracts and EOs of O. basilicum.*

	EtOH 100%	EtOH 70%	EtOH 30%	EtOH 5%	EOs
DPPH(IC_{50} µg/mL)	740±5.3^{A}	420±9.4^{D}	600±34.1^{B}	560±12.7 ^{C}	200±10.4 ^{E}
ABTS(IC_{50} µg/mL)	620± 14.6^{B}	380±5.8^{C}	660±2.4^{A}	600±44.2 ^{B}	300±7.9 ^{C}
FRAP(EC_{50} µg/mL)	1250± 12.2^{A}	950±17.7^{B}	1000±45.1^{B}	1040±12.6 ^{B}	540±33.2 ^{C}
BCBT(IC_{50} µg/mL)	1600±1.8 ^{A}	1300± 11.1^{B}	1200±18.9^{C}	1300±50.7 ^{B}	640±5.9 ^{D}

Means followed by the same letter are not significantly different at P ≤0.05.

3.4. Effects of Solvent Polarity and Essential Oil on Antibacterial Activity

The present study was investigated to determine the anti-bacterial activity of eluted ethanol-water mixtures of decreasing polarity and EO from *O. basilicum* against some pathogenic bacteria including *E.coli, P. aeruginosa* , *K. pneumonia*, *S. aureus, M. luteus* and *E. faecalis*,.

As shown by Table 4, the most relevant antibacterial activities were registered for EO, which effectively inhibited the growth of all bacteria with an inhibition diameter >15 mm. This ability seemed superior to that of EtOH 70%, especially against $Gram^{+-}$ bacteria. In addition, EtOH 30% and EtOH 5% displayed also an appreciable antibacterial activity especially against *E. faecalis* (IZ =19 and 9 mm, respectively) and *S. aureus* (IZ =17 and 9 mm, respectively) ; while EtOH 100% was inactive against $Gram^{-}$.

Following the same tendency, the potent antifungal activity was detected in EOs solution followed by EtOH 70%. According to Chimnoi et al. (2018) [12], 0.015–8.00 mg/ml of *O. gratissimum* essential oil extract caused rapid inhibition of *Escherichia coli* and *S. typhimurium*. In the report of Joshi (2013) [30] the test of the antibacterial activity from *O. gratissimum* ‘s essential oils and its primary ingredient, eugenol, revealed potent antibacterial activity against *Klebsiella pneumoniae*, *Serratia marcescens* and

E. coli. Reports from other workers also support the present study [31]. Indeed, it was reported that ethanolic extract of *O. basilicum* showed a notworthy inhibitory activity for Gram positive and negative bacteria. In the same line, Adiguzel et al. (2005) [32] proved that EtOH extract of *O.basilicum* has a strong antibacterial effect against nine strains from *Acinetobacter genera, Bacillus*. *Escherichia* and *Staphylococcus aureus*. In *vivo* investigation of Nakamura et al. (2004) [33] of antifungal activity of *O. gratissimum* essential oil against several Candida species demonstrated the higher fungicidal activity against *Candida albicans*, *Candida Krusei*, *Candida parapsilosis*, and *Candida tropicalis.*

Table4. *Antibacterial and antifungal activity of eluted ethanolic extracts and EOs from O. basilicum.*

Microbial strains	***EtOH 100%***	***EtOH 70%***	***EtOH 30%***	***EtOH 5%***	***EO diluted in DMSO***	***Genta***
Echerichia coli ATCC 35218	-	22 ± 0.4^{B}	14 ± 0.7^{C}	12 ± 0.6^{D}	29 ± 0.5^{A}	35
Pseudomonas aeruginosa ATCC 27853	-	17 ± 0.3^{B}	15 ± 0.1^{C}	9 ± 0.3^{D}	23 ± 0.2^{A}	27
Klebsiellapneumonia ATCC 4352	-	16 ± 0.1^{B}	10 ± 0.9^{C}	6 ± 0.5^{D}	19 ± 0.5^{A}	22
Enterococcusfaecalis ATCC 29212	5 ± 0.5^{E}	24 ± 0.2^{B}	19 ± 0.4^{C}	9 ± 0.5^{D}	29 ± 0.6^{A}	27
Micrococcusluteus ATCC 4698	4 ± 0.2^{E}	15 ± 0.7^{B}	12 ± 0.9^{C}	7 ± 0.7^{D}	20 ± 0.4^{A}	22
Staphylococcus aureus ATCC 25923	5 ± 0.4^{E}	19 ± 0.5^{B}	17 ± 0.1^{C}	9 ± 0.1^{D}	25 ± 0.3^{A}	27
Candida albicans ATCC 90028	2 ± 0.4^{E}	14 ± 0.4^{B}	10 ± 0.1^{C}	4 ± 0.2^{D}	16 ± 0.3^{A}	19
Candida glabrata ATCC 90030	3 ± 0.3^{E}	17 ± 0.2^{B}	10 ± 0.7^{C}	10 ± 0.5^{D}	19 ± 0.7^{A}	24
Candida krusei ATCC 6258	2 ± 0.6^{E}	19 ± 0.6^{B}	9 ± 0.5^{C}	12 ± 0.7^{D}	25 ± 0.6^{A}	27

Gentamycin: positive control

DMSO: negative control

No antimicrobial activity (na). inhibition zone < 1 mm. Weak antimicrobial activity (w). inhibition zone = 1 mm. Slight antimicrobial

activity. inhibition zone 2–3 mm. Moderate antimicrobial activity. inhibition zone 4–5 mm. High antimicrobial activity. inhibition zone 6–9 mm. Strong antimicrobial activity.inhibition zone > 9 mm. For all bacteria. the control (+) Gentamycin was use at 10 IU

3.5. Effects of Solvent Polarity and Essential Oil on Anti-Inflammatory Activity

The ability of eluted ethanol-water mixtures of decreasing polarity and EO from *O.basilicum* to inhibit cellular $NO^{\cdot}$ generation was estimated using LPS-stimulated RAW 264.7 macrophages. As presented in Figure 2, EO from *O.basilicum* exhibited particularly strong ability to inhibit LPS-induced NO secretion at concentrations ranging from 5 to 25µg/mL with IC_{50} values of 20 mg/mL. In comparison, L-NAME (a NO synthase inhibitor used as positive control) inhibited NO release by 45.7% at 67.4 mg/mL. Results revealed also that NO inhibition capacity of EtOH70%, EtOH30%, EtOH 100% EtOH 5% and EtOH30% extracts is noteworthy, with an inhibition percentage exceed 50%.

According to Ben Mansour et al. (2020) [11] the prevalence of rosmarinic acid compounds could partake in the inhibition of nitrite production from LPS-stimulated macrophages, thus modulate the expression of pro-inflammatory enzymes iNOS implicated in pleiotropic functions during inflammation. *Ocimum basilicum* L crude methanolic extract suppressed the induction of iNOS and the subsequent production of NO in LPS-stimulated RAW 264.7 macrophage cells [34].

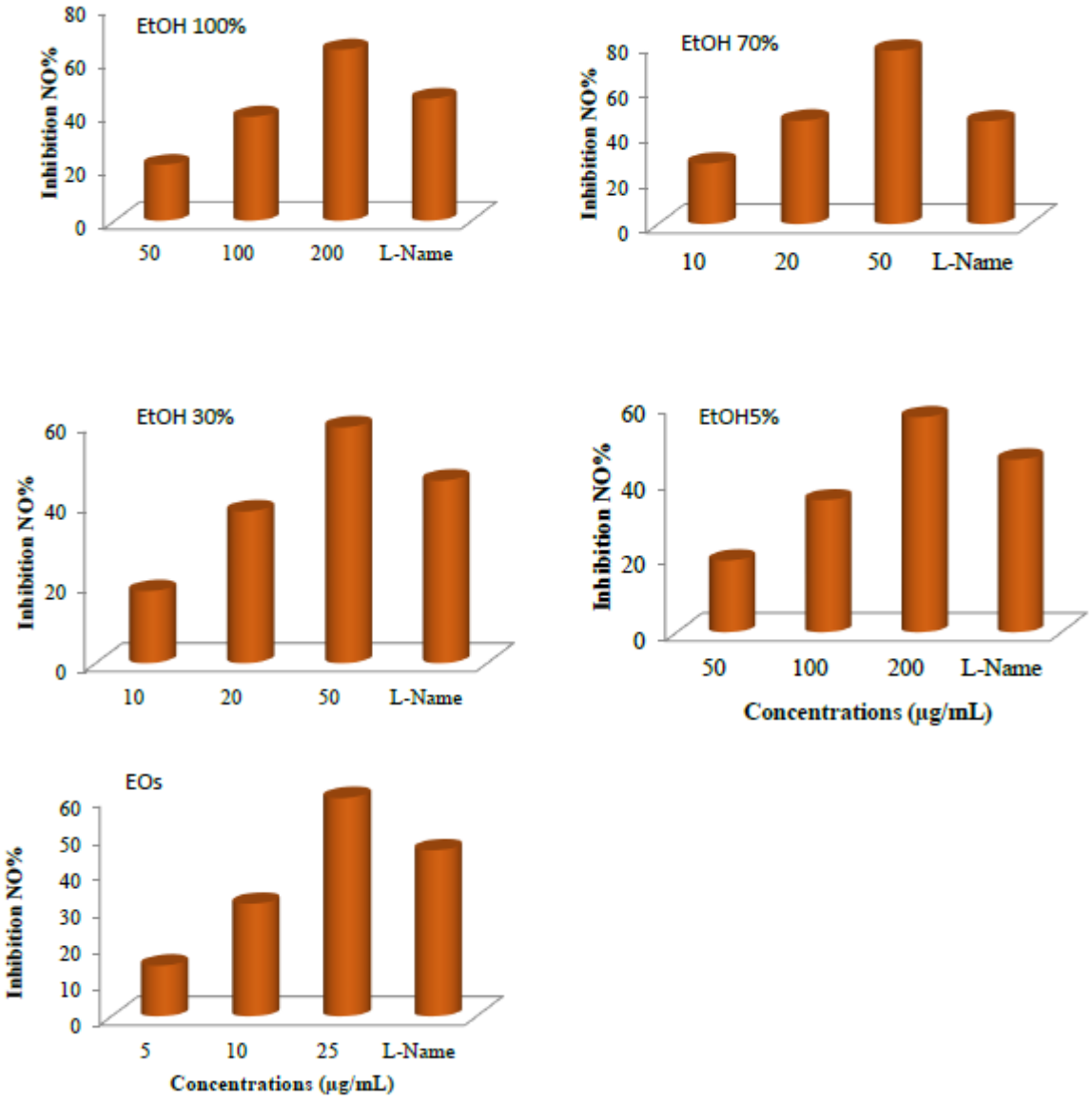

Figure2. *Anti-inflammatory activity of of eluted ethanolic extracts and EOs of O. basilicum*

4. CONCLUSION

Ethanol 70% seems to be recommended as a green solvent for phenolic extraction as well as for the appreciation of antioxidant activities in *O. basilicum*. Essential oil contributes equally as effective solution as source of antioxydants with health benefits.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGEMENT

This work was supported by the Tunisian Ministry of Higher Education. Scientific Research and Information and Communication Technologies (LR15CBBC06).

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Citation: *Mariem Saada et al, (2021). "Antioxidant, antimicrobial and anti-inflammatory activities of ethanol extracts and essential oil from the Tunisian glycophyte Ocimum basilicum L.". International Journal of Medicinal Plants and Natural Products (IJMPNP), 7(4), pp.1-11. https://doi.org/ 10.20431 /2454-7999.0704001*