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Effect of Hemicellulase on Extraction of Essential Oil from Algerian *Artemisia campestris*

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Abstract**—**Effect of enzyme on the yield and chemical composition of *Artemisia campestris* essential oil is reported in the present study. It was demonstrated that enzyme facilitated the extraction of essential oil with increase in oil yield and did not affect any noticeable change in flavour profile of the volatile oil. Essential oil was tested for antibacterial activity using *Escherichia coli*; which was extremely sensitive against control with the largest inhibition (29mm), whereas *Staphylococcus aureus* was the most sensitive against essential oil obtained from enzymatic pre-treatment with the largest inhibition zone (25mm). The antioxidant activity of the essential oil with hemicellulase pre-treatment (EO2) and control sample (EO1) was determined through reducing power. It was significantly lower than the standard drug (vitamin C) in this order: vitamin C>EO2>EO1.

Keywords**—***Artemisia campestris*, enzyme pre-treatment, hemicellulase, antibacterial activity, antioxidant activity.

I. Introduction

THE genus *Artemisia* (commonly wormwood or sagebrush) is one of the largest and most widely distributed genera of the family *Asteraceae* with approximately 500 species occurring through the world, growing in the northern hemisphere, south America, south Africa and pacific islands [1]-[3]. This genus is an important source of biological compounds for anti-inflammatory, antirheumatic, insecticides, fungicides, allelopathic and antibacterial activities [4]. *Artemisia campestris* is one of 11 species found in Algeria. It is an aromatic herbaceous annual plant largely used in traditional medicine against a variety of diseases. It has been used as febrifuge, vermifuge, against digestive troubles, gastric ulcer, and menstrual pain [5].

Essential oils (EOs) are aromatic oily liquids, volatiles, complex natural compounds extracted from various aromatic plants generally localized in temperate to warm countries. It has long been recognised since antiquity to possess biological activities, including antibacterial, antioxidant, antifungal, antiviral, antiparasitic, and insecticidal properties [6], [7]. Essential oils are defined as products obtained from raw plant material that must be isolated by physical methods such as distillation (steam, steam/water and water), expression (also known as cold pressing for citrus peel oils), or dry distillation of natural materials [8].

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In recent years, alternative processes have been developed in order to improve the recovery of natural products from plant materials. Enzyme-assisted extraction (EAE) has been successfully accepted as a potential alternative for natural products extraction with the advantages of environmental-compatibility and high efficiency to enhance the release and recovery of bioactive compounds from plants and simple operational processes. Enzymes have been used particularly for the treatment of plant material prior to conventional extraction methods. They have the ability to degrade or disrupt cell walls and membranes, thus enabling better release and more efficient extraction of bioactives [9]. Application of enzyme pre-treatment for the extraction of essential oils was investigated by [10]-[12]. The effect of enzymatic pre-treatment on the yield and chemical composition of the essential oil has been reported for some species such as garlic, celery, cumin seeds [12] and for *Fructus forsythia* [13], *Thymus capitatus* and *Rosmarinus officinalis* leaves [14]. It was noticed a significant increase in the yield of the extracted essential oil with a slight change in flavour profile or physico-chemical properties of the oil.

In the present study, hemicellulase was used for the pre-treatment of *Artemisia campestris* leaves which was subject to steam distillation. The effect of the enzyme pre-treatment on the yield, chemical composition, antibacterial, and antioxidant activities of *Artemisia campestris* essential oil was studied and compared to the essential oil obtained by the conventional method (untreated).

II. Material and Methods

A. Plant Material and Reagents

Artemisia campestris L. has been harvested in the highlands region located at 250 km south of Algiers at altitude of 1000 meters. Identification and systematic of the plant have been confirmed in the laboratory of botany (University of Blida, Algeria). Hemicellulase E.C.3.1.173 was purchased from Sigma-Aldrich (USA). Anhydrous sodium sulphate (Na_2SO_4) was obtained from Fluka chemicals; citric acid was obtained from (VWR BDH Prolabo, CE). Microorganisms were obtained from Saidal (Pharmaceutical Company, Algeria).

B. Enzyme Pre-Treatment

100 g of dried leaves of *Artemisia campestris* were mixed with 500 mL of distilled water containing 10 mg of hemicellulase, followed by the adjustement of pH to 4.5-5.0 using citric acid. The mixture was perfectly mixed and incubated at 45°C for 1h then subjected to steam distillation for the extraction of essential oil. In a control experiment,

sample of 100 g of dried leaves of *Artemisia campestris* without enzyme pre-treatment was subjected to steam distillation. In both cases, distillation was carried out for 3 H.

C. Isolation of Essential Oil

The essential oil was extracted by steam distillation. The collected oil was dried over anhydrous sodium sulphate, weighed, and stored at 4°C for further analysis.

The oil was collected and the yield of extracted essential oil (η) was calculated as follows: It is expressed in percent (%):

$$\eta(\%) = (W_{EO}/W_{Art})*100$$

where W_{EO} is the essential oil weight (g) and W_{Art} is the *Artemisia* leaves powder (g)

D. Gas Chromatography-Mass Spectrometry Identification

The essential oils were analysed by gas chromatography coupled to mass spectrometry (GC-MS). GC-MS analyses were carried out using a Hewlet Packard 6890A gas chromatograph coupled to a 5973i mass spectrometer with a HP5-MS column 30m x 0.25 mm x 0.25 µm. GC-MS spectra were obtained using the following conditions: Inlet temperature: 250°C, carrier gas: He; flow rate: 0.5 mL/min; Injection mode: Split (20:1); Injection volume: 0.2 µL. The oven used temperature program was 60°C for 8min; it was increased to 280°C at 2°C/min and held to 280°C for 10min. The ionisation mode was: electronic impact (EI) at 70 ev and ion source temperature of 230°C. Identification of compounds was confirmed by comparison of their GC retention time and mass spectra with those stored in the MS database (National Institute of Standards and Technology and Wiley libraries) and with mass spectra literature data [15]. A standard solution of n-alkanes (C_7-C_{26}) was measured to obtain the retention indices.

E. Antibacterial Activity

1. Bacterial Strains

The antibacterial activity of essential oil was tested against Gram negative bacteria: *Escherichia coli* (ATCC 10536) and Gram positive bacteria: *Staphyloccocus aureus* (ATCC6538).

2. Disc Diffusion Method

Antibacterial activity was carried out using the disc diffusion method [16]. The Muller Hinton (MH) agar was used as culture medium for the bacteria. 100 µL of standardized inoculum suspension was adjusted to 0.5 Mcfarland (10^7 to 10^8 CFU/mL) of tested microorganisms and spread on the MH media plate (90 mm diameter). Sterile paper discs (6mm in diameter/Whatman n°1) were impregnated with 20 µL of essential oil and deposited on plates, then incubated at 37°C for 24h. The inhibition zones (IZ) were measured and expressed in millimeters (mm).

F. Ferric Ions (Fe^{3+}) Reducing Antioxidant Power Assay

The FRAP method is based on a redox reaction in which an easily reduced oxidant (Fe^{3+}) is used in excess and antioxidants act as reductants [17]. This technique was used to measure the reducing capacity of *Artemisia campestris* essential oil, according to the method of [18]. The different concentrations of essential oil were mixed with 2.5mL of phosphate buffer solution (0.2M, pH 6.6) and 2.5 mL of potassium ferricyanide solution ($K_3Fe(CN)_6$, 1%). The mixture was incubated at 50°C for 20 min, and then 2.5mL of trichloroacetic acid (10%) was added to the mixture which was then centrifuged at 300 rpm for 10min. 2.5 ml of upper layer solution was mixed with 2.5mL of distilled water and 0.5mL of ferric chloride solution (0.1%) and the absorbance was measured at 700nm in spectrophotometer**.** Ascorbic acid was used as positive control. Increased absorbance of the reaction mixture indicates an increase of reduction capability [19].

III. Results and Discussion

A. Extraction and Yields of Essential Oils

Effect of hemicellulase pre-treatment has shown an increase in the yield of essential oil compared with the control (untreated) sample. The use of hemicellulase pre-treatment of dried leaves of *Artemisia campestris* improved the release of essential oil obtained by steam distillation. The yield of extraction varies from 0.88% for control sample to 1.5% for the treated sample. This behaviour can be explained by the disruption of the structure and integrity of the cell wall, enhancing by this means the extraction of essential oil [20].

B. Chemical Composition

Effect of hemicellulase on quality of essential oil was studied (Table I). Analysis by CG-MS of essential oil extrated by steam distillation from *Artemisia campestris* treated with hemicellulase pretreatment (EO2) and untreated (control sample: EO1) showed the presence of α-Pinene (19.9%), (19.6%) respectively as major compounds, followed by germacrene D (7.1%), (10.6%), α-Copaene (8.2%), (8.1%), α-pinene (6.1%), (6.2%), and Spathulenol (5.4%), (5.1%) respectively. In literature, a number of studies on the chemical composition of *Artemisia campestris* essential oils reported that α-pinene, β-pinene, or (Z,E) farnesol, cedrol, verbenone, or camphor, 1,2-dehydro acenaphthylene were the dominants compounds [3]. It has been shown the presence of β-pinene (24.0-49.8%) and (6.9-57.2%) as major constituents of essential oils extracted from the specie grown in Tunisia and Italy and α-pinene as the main component in the oil obtained from Tunisia (5.9-12.5%) and Algeria (18%) [21]. Belhattab et al. [5] reported the presence of α-terpinene and α-pinene (18.8 and 18.4% respectively) and camphor (9%) in *Artemisia campestris* essential oil extracted from south Algeria. Analysis of the chemical composition of *Artemisia* essential oil extracted from plant grown in showed (Z,E)-farnesol (10.3%), cedrol (5.4%) and verbenone (3.8%) as the main components. which suggest that different chemotypes may exist in Algeria [22].

C. Antibacterial Activity

The in-vitro antibacterial activity of *Artemisia campestris* essential oil was evaluated by the measure of inhibition zone

diameter around the discs containing the samples to be tested to the pathogenic bacteria (*Escherichia coli, Staphylococcus aureus*) after 24 h of incubation at a temperature of 37°C. Inhibition zones can be symbolized by signs according to susceptibility testing of strains via the essential oil: i) not sensitive (-) or resistant: diameter<8mm. ii) sensitive (+): diameter comprised between 8 and 14 mm. iii) highly sensitive (++): diameter between 14 and 19 mm. iv) extremely sensitive (+++): the diameter>20mm [23], [24].

The obtained qualitative results (Table II) showed a variable degree of the antibacterial activity against the strains tested with the essential oil obtained from hemicellulase pre-treatment and control (untreated). Essential oil was active against the tested microorganisms. *Escherichia coli* was extremely sensitive against control with the largest inhibition (29mm). *Staphylococcus aureus* was also extremely sensitive against essential oil obtained from enzyme pre-treatment with the largest inhibition zone (25mm).

As reported in the literature, Tunisian *Artemisia campestris* essential oil showed a strong effect on *Escherichia coli* [25], while it was the most sensitive bacteria in our samples. Baykan Erel et al. [3] reported the antimicrobial property of *Artemisia campestris* essential oil from western Anatolia. It has been shown that the medicinally important pathogen *Staphylococcus aureus* was the most sensitive bacteria to essential oil. This difference might be due to the different essential oil compositions.

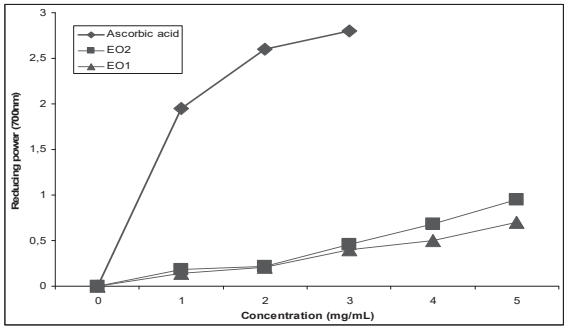

Fig. 1 Ferric reducing activity of *Artemisia campestris* essential oil and vitamin C

D. Reducing Power Assay

The reducing capacity of a compound may serve as an indicator of its potential antioxidant activity [26]. Fig. 1 shows the concentration-response curves for the reducing power of vitamin C, plant essential oil obtained from enzyme treatment (EO2) and control sample (EO1). The antioxidant activity of the essential oil which was reflected through the reduction of Fe^{+3} to Fe^{+2}, was increased with increasing concentration. The antioxidant activity of both samples (EO1) and (EO2) were significantly lower than the standard drug (Ascorbic acid or Vitamin C). At 5 mg/mL, EO2 showed a reducing power of 0.95 higher than EO1 (0.70) in terms of absorbance values at 700 nm. The antioxidant activity of the essential oil with hemicellulase pre-treatment (EO2) and control sample (EO1) was significantly lower than the standard drug in this order: vitamin C>EO2>EO1.

TABLE I
CHEMICAL COMPOSITION OF *ARTEMISIA CAMPESTRIS* ESSENTIAL OIL

Compounds[a]	RI[b]	EO1 (%)	EO2(%)
α-Thujene	931	0.07	0.05
α-Pinene	939	19.6	19.9
Fenchene	951	0.09	0.1
Camphene	953	1.5	1.1
Sabinene	977	0.1	0.1
β-Pinene	980	6.4	6.2
Myrcene	990	2.1	2.2
α-Phellandrene	1005	0.1	0.4
α-Terpinene	1019	0.5	0.7
p-Cymene	1028	1.3	0.6
Limonene	1032	4.4	4.1
1,8-Cineole	1035	0.2	0.3
cis-β-Ocimene	1041	1.3	1.7
trans-β-Ocimene	1050	1.9	2.1
γ-Terpinene	1062	1.1	1.2
Terpinolene	1090	0.15	0.11
3-Ethyl-O-Xylene	1113	0.15	0.06
allo-Ocimene	1132	0.2	0.1
Camphor	1141	0.09	0.1
trans-Pinocarveol	1143	0.08	0.1
cis-Verbenol	1145	0.09	0,1
trans-Verbenol	1150	0.8	1.2
Pinocarvone	1160	0.2	0.1
Verbenone	1164	2.8	3.1
Borneol	1168	0.1	0.2
Terpinen-4-ol	1180	0.3	0.9
p-Cymene-8-ol	1187	-	0.1
α-Terpineol	1192	1.1	1.4
Myrtenol	1197	-	0.9
trans-Carveole	1223	0.2	0.1
Geraniol	1257	0.1	0.1
cis-Chrysanthenyl acetate	1264	0.3	0.3
Bornyl acetate	1289	0.1	0.09
trans-Sabinyl acetate	1295	0.4	0.2
α-Terpenyl acetate	1340	2.5	2.1
α-Longipinene	1347	0.3	0.1
α-Cubebene	1353	0.2	0.1
Eugenol	1359	-	0.1
Geranyl acetate	1370	1.3	1.1
α-Copaene	1376	8.2	8.1
β-Bourbonene	1381	0.1	0.2
β-Elemene	1393	0.2	0.1
α-Gurjunene	1400	-	0.1
β-Caryophyllene	1420	3.2	3.1
Aromadendrene	1440	0.1	0.2
α-Humulene	1450	0.1	0.1
Alloaromadendrene	1461	0.11	0.2
GermacreneD	1471	10.6	7.1
α-Curcumene	1487	0.6	3.1
α-Muurolene	1495	0.3	0.5
Bicyclogermacrene	1496	0.1	0.8
E,E,α-Farnesene	1505	0.8	0.7
α-Amorphene	1508	-	0.1
γ-Cadinene	1509	0.35	0.1
E,E,α-Farnesene	1516	0.6	0.4
δ-Cadinene	1520	0.45	0.3
Germacrene B	1540	0.3	0.1
E-Nerolidol	1566	0.1	0.32
Spathulenol	1577	5.1	5.4
Caryophyllene oxide	1583	0.2	0.1
Geranyl isovalerate	1613	0.9	1.1
α-Cadinol	1644	1.8	2.6
β-Eudesmol	1650	3.1	3.8
Vulgarol B	1652	0.3	0.2
α-Eudesmol	1663	0.2	0.1

[a]Compounds listed in order of their RI
[b]RI Kovat's retention index

TABLE II
DIAMETER OF THE INHIBITION ZONE

Strains	Gram	EO1 (control)	EO2 (enzyme pre-treatment)
Escherichia coli	-	29 mm	17 mm
Staphylococcus aureus	+	12 mm	25 mm

IV. CONCLUSION

Enzymatic pre-treatment can be considered as a novel and environmentally friendly extraction technique. Hemicellulase was employed with the aim to find the best yield and improve the quality of essential oil extracted by steam distillation. An increase in yield, from 0.88 to 1.5%, was observed after the use of hemicellulase enzyme. Enzymatic pre-treatment did not affect any noticeable change on chemical composition of the volatile oil. Essential oil was active against the tested micro-organisms. *Escherichia coli* was extremely sensitive against control. *Staphylococcus aureus* was the most sensitive against essential oil obtained from enzyme pre-treatment. Antioxidant activity of the essential oil with hemicellulase pre-treatment (EO2) and control sample (EO1) was significantly lower than that of the standard drug (vitamin C).

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