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Comparative study of the chemical composition, antifungal and antibacterial activities of *Juniperus oxycedrus* (leaves and streams) essential oils from Algeria

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Abstract

We've choosen this work to show the influence of extraction method on the chemical composition of the extraction essential oil. The extraction of essential oil of streams and leaves from north western Algeria (mostaganem) is performed using both processes training with water vapor (TWV) and hydro-distillation (HD); which is identified by gas chromatography coupled to mass spectrometer (GC-MS) and to evaluate their antifungal and antibacterial activities analysis. Which show that (GC-MS) led to the identification of 35 composents using both methods (HD) was found to be the best process for extraction of this vegetal substance following composition terpinene4-ol (17,48%), sabinene (14,93%), alpha pinene (14,85%). (HD) oil is the best antifungal as a minimum concentration (20mg/ml), and (TWV) oil had a higher inibitory effect in the antibacterial test as minimum concentration (10mg/ml). Essential oil of *juniperus oxycedrus* has a good antifungal and antibacterial activity and important source in pharmacetic.

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Introduction

Juniperus oxycedrus (Cupressaceae) also called Juniper masterpiece is a wild shrub in stony places in the Mediterranean (ADAMSR.P1998; ADAMSR. P2004) this species can reach up to 14 m high, but it is usually in the form of a shrub, 1 to 2 m; Sometimes the leaves of the Cade are shaped needles that measure up to 25 mm.

The leaves grow whorled by three on recent shoots. Male and female flowers grow on different trees: it takes a male tree and a female tree to fruit. Brown Red and matures at the end of two years. It measures between 12 and 15 mm (Montagne,P,1999).

Essential oils of this species are used in the area popular for their antiseptic properties and disinfectant medicine. Regulation of the balance of the scalp; Vermifuge; Anti-parasitic (Leung and Foster,1996)

Objectives of our study

Our goal in this work is to study the characterized of the chemical composition of the essential oils of the leaves and streams of Algerian species; and study their activities: antifungal and antibacterial study fits into the overall context of a local product that can improve the use of aromatic and medicinal plants Algerian in general.

Material and methods

Plant materials

Palm (stems and leaves) of Juniperus oxycedrus has been collected during the month of May 2010 in the drilled of wilis in mostaganem (North of Algeria).

Extraction of essential oils

Extraction of essential oils has been carried out by two different distillation (ANONYME, 2002) techniques was accomplished using a device of type Clevenger (Clevenger JF,1928; Kumar and al 2010) and training water vapor has on a device of type alembic. 100 g of dry raw was treated to the first method and 500g for the second process. The duration of extraction is of the order of 3 hours.

GC–MS analyses

Chromatographic analysis was carried out using an Agilent 5973 GC - MS coupled with an Agilent 6800, equipped with an injector split whose report of leak is (1 70) at 250 ° C.

The analytical Conditions have been set as follows: Agilent HP - 5ms capillary column (30 m x 0.25 mm, df = 0.25 Um), program of temperature: 60 ° - 250 ° C to 2 ° C / min, mobile phase: carrier gas was helium to 0.5 ml / min. The fragmentation is performed by impact electronic a70 eV, the digitized mass range: 34-450 uma.

Source and quadruples temperatures have been set at 230 ° C and 150 ° C, respectively. The identification of the components were made on the basis of the retention by chromatography indices and by comparison of the Spectra recorded with libraries of calculated data (wiley7n.l and NIST02. (L).

Microbiological process

Evaluation of antifungal and antibacterial activity we used the technique of dispersion of essential oils in the agar to 0.2% (Remmal and al 1993 ; Satrani and al 2001) and used the methanol for the dilution of the extract.

The final concentrations of essential oils are: 5,10,20,40,80 (mg/ml). Witnesses, made up of the growing medium more single agar-agar to 0.2% solution, are also prepared. 2 ml of each concentration have been added to the tubes a test containing nutrient agar for bacteria and the PDA for molds. They are then sterilized a autoclaving (20 minutes at 121 ° c.), cooled at 45 ° C.

The mixtures were sunk on the boxes dish. After, the disks of mycelium of each mold of 5mm in diameter cut device of a culture of 7 days are inoculated in the center of boxes and then incubated at 25 ° c± 2 for seven days.

The incubation temperature is 37 ° C for 24 hours for the bacteria each test is repeated three times to minimize experimental error.

Results and discussion

Yield and chemical compositio

Method of hydro-distillation provided a performance in essential oils of about 0.12 % against only 0.07% for those in the training method has the steam. However, this rate is relatively high compared to this rapport of other studies (Table 1).

Table 1. Relatively high compared to this rapport of other studies.

Reference	Mansouri *et al.*,2010	Montagne,1999	Remmal, 1993	Our study
Country	Maroco	Libanon	Spain	Algeria
Rendement (%)	0,15	0,72	1,14	0,12

The results of the analyses by CG and CG/MS of essential oils extracted from twigs of *Juniperus oxycedrus* collected in the North of Algeria are present in (Fig. 1&2) and (Table 2).

Table 2. Essential oils chemistry of the *Juniperus oxycedrus* means Mostaganem Algeria.

N	KI	Compounds	H D Oil(%)	TWV Oil (%)
01	920	Cyclooctadiene	-	0.26
02	929	A-Thurjene	2.27	2.69
03	936	A-Pinene	14.85	14.01
04	947	Camphene	0.11	-
05	973	Sabinene	14.93	18.15
06	993	B-Myrcene	1.05	1.61
07	1010	4-Carene	0.17	1.06
08	1010	Δ -3 Carène	0.74	-
09	1031	Limonene	2.62	3.98
10	1038	P-Cymene	9.12	12.25
11	1059	Δ-Terpinene	2.71	3.95
12	1060	A- Terpinene	1.13	1.70
13	1087	A- Terpinolene	-	1.31
14	1088	Terpinene-4-Ol	17.48	8.45
15	1122	Cis-P-2-Menthen-1-Ol	1.02	-
16	1127	A -Campholenal	-	0.92
17	1158	Sabina Ketone	1 .02	-
18	1188	Verbenol	0.85	-
19	1205	Verbenone	0.54	-
20	1218	Trans-Carveol	0.49	-
21	1283	P-Cymene-7-Ol	0.42	0 .15
22	1332	Adamantane	1.38	-
23	1383	B-Bourbonene	-	0.40
24	1422	Coryophyllene	-	1.84
25	1482	Germacrene-D	-	1.37
26	1523	Δ-Cadinene	-	0.32
27	1570	Caryophyllene Oxyde	-	0.90
28	1584	Bicyclo[2.2.2]Oct-2-Ene, 1,2,3,6-Tetramethyl	-	0.24
29	1602	Dodecenylacetate	-	1.56
30	1632	Trans-Pinocarveol	0.68	0.16
31	1683	Sabinol	1.75	-
32	1702	Aromadendrene Oxyde	-	0.62
33	1707	Farnesol	-	5.22
34	1824	P-Mentha-1,5-Dien-7-Ol	1.182	0.47
35	1955	Pimaradiene	-	0.25
36		Fenchyl Alcool	0.80	0.83
37		Sekisanin	-	1.280
38		Cis-Piperitol	0.31	-
39		2,5-Diethylfuran	0.27	-
40		7-Hydroxynorbornadiene	0.20	-
41		Benzene, (3-Methyl-2-Butenyl)	-	0.81
42		Tridiconone	-	1.07

The composition of the essential oils extracted from the HD is different from the extracted composition of the TWV, despite the use of the same sample, however the similarity found in essential oils extracted from both methods often in the following compositions (Terpinene, Sabinene, Alpha Pinene) except that they are different in some components for example (Farnesal5, 22%; methyldesoxylithofellate6, 43%) located in the composition from the EVD and (1-Bromoadamantane, 1, 38% (Sabinol 1.75%) located in the extracted composition of the HD.

Table 3. Antifungal and antibacterial activity of essential oils extracted from the leaves and stems of *Juniperus Oxycedrus.*

Concentrations (mg/mL)		5		10		20		40		80	
A samples		TWV	HD	TWV	HD	TWV	HD	TWV	HD	TWV	HD
The Molds	*Botritice cineria*	+	+	+	+	+	–	–	–	–	–
	Fusarium oxysporum	+	+	+	+	+	+	+	+	–	+
	Aspergillus Barasilians	+	+	+	+	+	+	+	+	+	–
	Ascochyta rabiei	+	+	+	+	+	+	–	+	–	+
The bacteri	*Escherichia coli*	+	+	–	+	–	+	+	–	–	–
	Staphylococcus aureus	+	+	+	–	–	–	–	–	–	–

(+) = growth / development; (–) = inhibition / Inhibiti.

The composition of the essential oil, which is found in the leaves and stems of the *Juniperus oxycedrus*, picked in the forest of WILLIS in the Town of Mostaganem (Algeria), is different to the one obtained previously the *Juniperus oxycedrus* and that obtained by N. (Mansouri *et al.*,2010)-Atlas Maroc studied by Velasco Nene Gueruela (Velasco *et al.*, 2003).

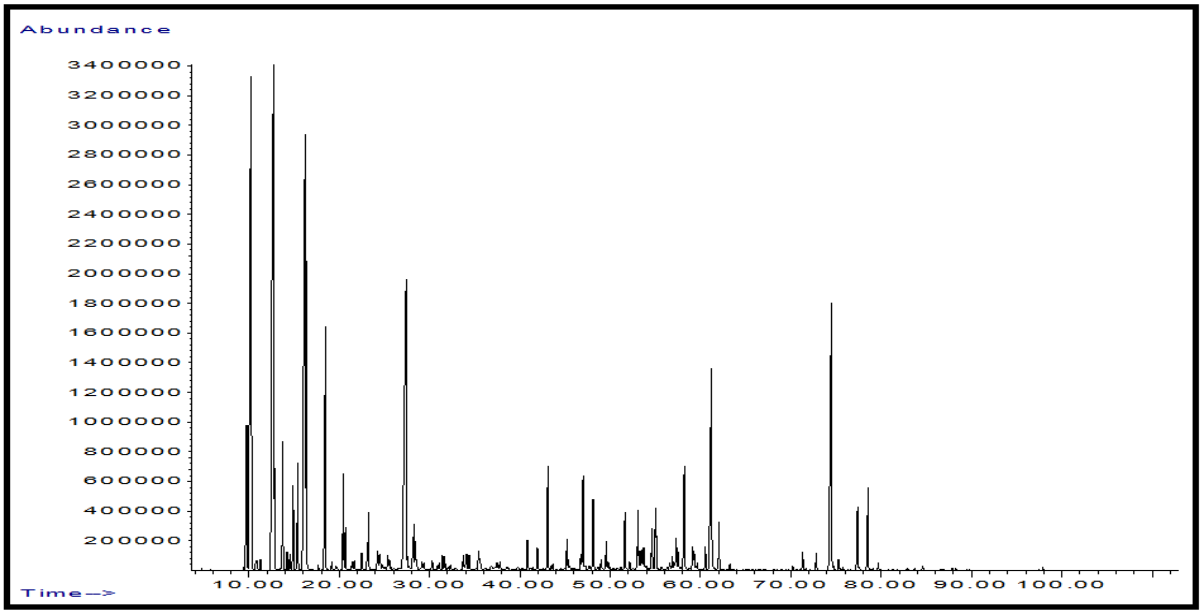

Fig. 1. Essential oil of Juniperus oxycedrus extracted by entrainment a steam.

The results obtained concerning: the method undertaken for the extraction of the oils, and the geographical position of the sample.

Antifungal and antibacterial activities

The results of the antifungal activity of essential oils extracted from the leaves and stems of *Juniperus*

Oxycedrus at the level of the Town of Mostaganem are grouped in the (Table3). Essential oils extracted using a process TWV have was most active inhibited mold (*Fusarium oxysporum, Aspergillus Barasilian*) at a rate of 80 mg/ml concentration, this result does not apply on the second method because it was not active for these two molds.

The most sensitive mold was *Botritice cineria*, whose growth inhibition is 20 mg/ml of the essential oils extracted using a process HD and 40 mg/ml of the essential oils extracted using a process TWV, also as *Ascochyta rab* IEI to defeat 40 mg/ml of the essential oils extracted using a process TWV, and 80 mg/ml of the essential oils extracted using a HD process.

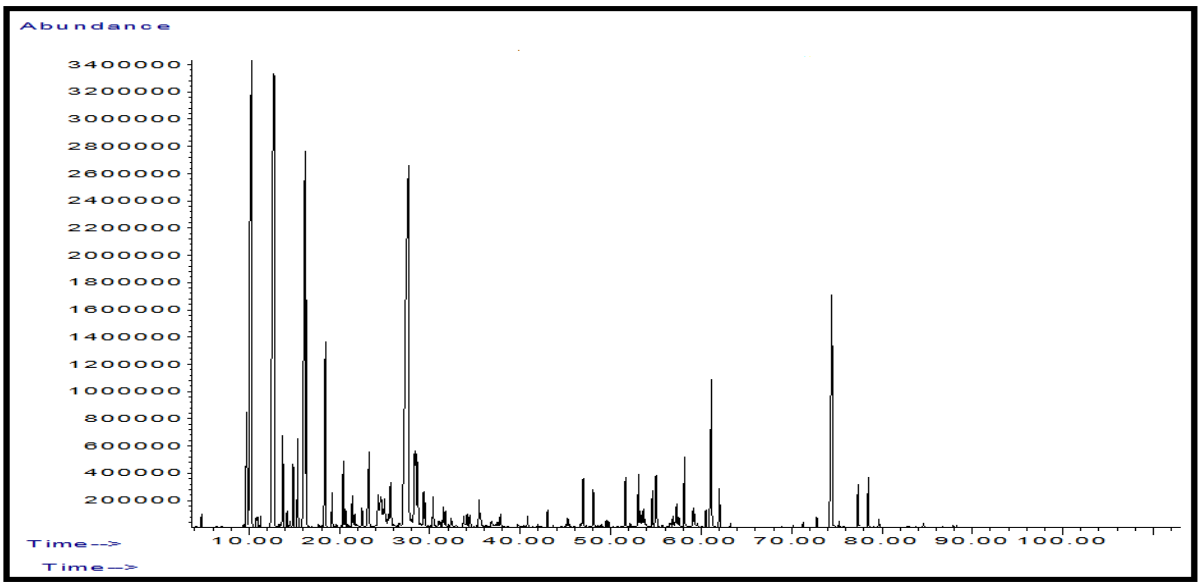

Fig. 2. Essential oil *Juniperus oxycedrus* extracted by hydro-distillation.

This antimicrobial activity observed for essential oils has been reporting that the α-pinene, which is made majority of *Juniperus oxycedrus*, present several biological activities. (Duke JA, 1998) .We notes that the essential oil of *Juniperus oxycedrus*, shows that it presents a good inhibitory activity against *Escherichia coli* and *Staphylococcus aureus*. (Clevenger JF,1928)

Conclusion

The comparative study of the essential oil of the streams and leaves of juniperus oxycedrus stem extracted by the two extraction method was identified more than 35 compounds by GC - MS. distillation was the best process for the extraction of oil juiniperus oxycedrus starkest, and composing them main was terpenene-4-ol follow-up of sabinene and α-pinene gasoline of the Juniper oxycedrea unenefficacite case against all fungi tested, she showed that she has a good antifungal activity that also regarded as an important source of information in the food industry and pharmacetic

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