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Chemical quality, antibacterial and antifungal activities of *Cotula cinerea* essential oil from South Morocco

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ABSTRACT

This work had done the object of the chemical quality study and the evaluation of antibacterial and antifungal activity of the *Cotula cinerea* essential oil from South Morocco. The essential oils obtained by hydrodistillation of the aerial part of the plant had been analyzed by GC/FID and GC/MS. The essence of *Cotula cinerea* is characterized by dominance of the 3-iso-thujanol followed by Santolina triene and camphor. The antimicrobial activity of *Cotula cinerea* essential oils had been estimated vis-à-vis of four bacterial strains, three molds and four reference woods rot fungi. The essential oil of this species showed a strong antimicrobial power against the tested microorganisms.

INTRODUCTION

Morocco, from its geographical location, constitutes a completely original natural frame offering a complete range of Mediterranean and Saharan bioclimates favoring a rich flora and varied with a very marked endemism. It occupies the second rank among the Mediterranean countries[8].

In Morocco, the medicinal and aromatic plants represent an important category of non-ligneous forest products. They involve a wide range of products which exist in spontaneous state, in forests and outside forests or in a cultures form. After having been considered, for a long time, as secondary products or menus products, the medicinal and aromatic plants had taken a considerable development in consideration of the constantly increased demand in the international market.

The numerous medicinal and aromatic plants encountered in different Moroccan regions possess well therapeutic virtues demonstrated by the experience. However, and in a general manner, the possibilities of curing the vegetable world, whatever be affirmed or potential, deserve to be justified by the scientific study. Besides, to bring scientific evidences to the activity of essential oil of some plants would be very useful in the increase of the economic value of these natural resources and the development of a strategy of management and preservation of these plants.

The state of the spontaneous flora in the regions of Moroccan Sahara as well as the relations between

humans and the plant species deserve a particular attention. Some species possess pharmacological properties, which confer them a medicinal interest. The natural remedies and especially medicinal plants had been, during a long time, the main or even the only recourse of the oral tradition to treat the pathologies, at the same time as a raw material for the modern medicine[3,4]. Among these plants, we quote *Cotula cinerea* L., syn. *Brocchia cinerea* Del., called locally " Rabrouba or Al Gartoufa ". It belongs to the family of *Asteraceae*[16,31]. The genus *Brocchia* Vis. had been described by Visiani[37] on the basis of the annual plant of *Cotula cinerea* Delile. It comprises more than 1,000 genera and 15,000 species spread over all continents and in the most various mediums[9]. The species *Cotula cinerea* is very encountered all over the Sahara, it grows in the ergs and the less sanded grounds in Moroccan Sahara's regions[9].

It is an annual plant of woolly aspect from 5 to 15 cm entirely tomentose. The stems are drawn up or diffuse. Green-whitish leaves and stems are covered with small dense hairs. The small leaves, entire thick and velvety are cut out into three to seven teeth or 'fingers' which are presented as a hand slightly closed. The flowers are small half pompoms yellow of gold at the tip of a short stem. It is a common Saharo-Arabic species in all Sahara and the desertic sandy places[31].

This species is widely used in Moroccan traditional medicine for its biological properties like the anti-inflammatory, analgesic, antiseptic, antibacterial, antipyretic activities and against the larvae's[19,24,27,28,29]. It can be used in the treatment of the stomach pains, the fever, the headaches, the migraine and cough[11]. Several pharmaceutical works had studied the antiparasitic and antimicrobial properties of some extracts of *Cotula cinerea*[17]. The extracts of this plant's leaves are effective against the pathogenic fungi and they have an insecticidal activity against insects larvae's[17].

This plant contains a variety of chemical compounds with therapeutic properties, such as : the flavonoids[15], the terpenes and the essential oils, which give to the plant its olfactory specificity[27].

It is within a contribution frame to the valorization of our Moroccan Sahara's natural heritage that this work is registered. According to our bibliographical investigations, the phytochimic study of the extracts of *Cotula cinerea* had done the object of several scientific works, however, the essential oils antifungal activity of this species against wood rot fungi has never been carried out before. Our current work aims at the chemical characterization and the evaluation of essential oils antibacterial and antifungal activities obtained from the Moroccan's areal parts of *Cotula cinerea*.

MATERIAL AND METHODS

Material plant

The *Cotula cinerea* (areal parts) samples had been collected during 2015 March month, in Dayt Salwane (Oued Sagia Al Hamra, Smara) of Moroccan Sahara region depending on AFNOR norm[2]. The plants had been taxonomicaled and identified at the Forestry Research Center in Rabat.

Studied microorganisms

Four bacteria (*Bacillus subtilis, Escherichia coli, Staphylococcus aureus* and *Micrococcus luteus*) : bacterial strains which are lots ATCC (American Type Culture Collection). They are maintained by subculture on nutrient agar favorable to their growth during 24 h in the obscurity and at 37°C.

Seven fungi of which three molds (*Asper gillusniger, Penicillium digitatum* and *Penicillium expansum*) are known by their high degrees to contaminate the foodstuffs and by their pathogenicities. For other fungi, they are fungal species, responsible of brown and white rot wood. They were chosen for the considerable damage which they cause to timber and derived products (*Gloeophyllum trabeum, Coniophora puteana, Poria placenta* and *Coriolus versicolor*).

The fungal strains and molds belong to the Mycotheque Collection of Microbiology Forestry Centre (Rabat, Morocco) Laboratory. They are regularly maintained by transplanting on the nutrient environment PDA (Potato Dextrose Agar).

Extraction of essential oils

The areal parts of *Cotula cinerea* essential oils were obtained by hydrodistillation during 180 min of extraction in a Clevenger type apparatus[13]. The yield samples of essential oil was determined after three hydrodistillations

of 200g dry material, and the moisture calculation by drying 20g of each sample during 4 h in a stove at 102°C. The obtained essential oil is dried on anhydrous sodium sulfate, and conserved at an obscurity of 4°C temperature.

Chromatographic analysis

The gas chromatography (GC) analysis were performed using a Hewlett Packard Gas Chromatographer (HP 6890) with electronic pressure control, equipped with a HP-5MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm), a FID detector set at 250 °C and using a H 2 /Air mixture, and a split-splitless injector set at 250 °C. The injection mode was split (split ratio: 1/50, flow rate: 66 ml min^{-1}) and the injected volume was about 1 µl. Nitrogen was used as carrier gas with a flow rate of 1.7 $ml.min^{-1}$. The column temperature was programmed from 50 to 200 °C at a heating rate of 4 °$C.min^{-1}$, during 5 min. The apparatus was controlled by a "Chemstation" computer system.

The gas chromatography/mass spectrometry (GC/MS) analysis were performed by a Hewlett-Packard Gas Chromatographer (HP 6890) coupled with a mass spectrometer (HP 5973). Fragmentation was performed by electron impact at 70 eV. The column used was HP-5MS (30 m x 0.25 mm, film thickness 0.25 µm). The carrier gas is helium whose flow is fixed at 1.5 $ml.min^{-1}$. The injection mode was split (split ratio: 1/70, flow rate112 ml min-1). The column temperature was programmed from 50 to 200 °C at a heating rate of 4 °$C.min^{-1}$, during 5 min. For the chromatographic analysis, essential oils were diluted in methanol (1/20 v/v).

The identification of the components is based on the comparison of their mass spectra (GC/MS) (Jilali & Sereshti, 2007), respective with spectra of the library (NIST 98), of the bibliography[1] and on the basis of calculation of Kovats indices[23]. Indeed, the index system is based on a notion of relative retention. It compares the retention of whatever product to that of a linear alkane. This system is applicable in gas chromatography to all compounds on all columns. By definition, it assigns an index of 800 to the linear alkane in C 8 (n-octane), 1000 to C 10 linear alkane (n-decane), and this, whatever the stationary phase, the length of column, the flow rate or the temperature. The KI are determined by injecting a mixture of C 9 to C 24 alkanes in the same operating conditions. They are calculated from the following equation :

$$Ik=\left[\frac{TR_x - TR_n}{TR_{n+1} - TR_n}+n\right]\times 100$$

Where in TR x is the retention time of the solute x, TR n and TR n+1 are the retention times of linear alkanes to n and n+1 carbon atoms and which frame the peak of the solute. The retention index KI or a compound A is independent from the flow rate, of the column length and of the injected amount (within a certain limit). The retention index of a compound A depends on the stationary phase and temperature.

In general, the technique of KI is widely used to identify the usual essential oils compounds, but it is insufficient to determine the total chemical composition. The IK tables specific to each product are proposed in the literature. They were developed using analyzes on different types of columns. These benchmark indices are compared to those calculated from our samples.

Microbiological procedure

The minimum inhibitory concentrations (MIC) of the essential oils were determined according to the method reported by Remmal and al[32], also by Satrani and al[34]. Because of the essential oil immiscibility with water and, therefore, to the cultural environment, an emulsification was realized thanks to an agar solution at 0.2%. It allowed to obtain, in the middle, a homogeneous distribution of essential oils and to make the higher maximum of compound/germ contact. Dilutions are prepared at 1/10e, 1/25e, 1/50e, 1/100e, 1/200e, 1/300e, 1/500e in this agar solution.

In test tubes, containing each 13.5 ml of solid environment TSA (Tryptic Soy Agar) for bacteria, and the PDA (Potato Dextrose Agar) for fungi, sterilized at the autoclave during 20 min at121 °C and cooled at45 °C, we add aseptically 1.5 ml of each dilution so as to obtain the final concentrations of 1/100, 1/250, 1/500, 1/1000, 1/2000, 1/3000, 1/5000 (v/v). We shake the tubes to disperse properly the essential oil in the cultural environment before pouring them into Petri dishes. Witnesses, containing the cultural environment and agar solution at 0.2 % alone, are equally prepared. The seeding is done by streaking with the help of a calibrated platinum loop to withdraw the same inoculums volume.

This latter is presented in the form of culture broth of 24 h for bacteria and in the form of a suspension in physiological water of spores resulting from a culture of 7 days in the PDA for fungi. The seeding is done, for these latters, by the fragments deposition of 1cm3 of diameter, taken from the periphery of a mycelia mat, and originating from a 7 days culture in the malt extract. The incubation is done at 37°C during 24 h for bacteria, and at 25°C during 7 days for fungi. Each test is repeated three times.

RESULTS AND DISCUSSION

Yields and chemical composition

The *Cotula cinerea* essential oil is viscous with olfactory characteristics which remind that of *Artemisia* and its yellowish color. Indeed, the essential oils coloring is very influenced by the oil chemical compounds nature[6].

The essential oil's average yield of *Cotula cinerea* had been expressed in ml per 100 g of the plant's dry vegetable aerial part. This yield is of 0.64 ± 0.02 %. This Moroccan Sahara's Dayt Salwane essential oil content of *C. cinerea* is high compared to that of Algerian South East region, which ranges from 0.08 % to 0.39 % depending on the flowering and fruiting stage[12]. It is also higher than that obtained for different parts of the Tunisian's, *Cotula coronopifolia* which ranged between 0.001 and 0.03 %[22]. However, this rate is lower than that provided by *C. cinerea* from Zagora's region (South Morocco) which is 0.87 %[17].

The *Cotula cinerea* essential oils chromatographic analysis of Dayt Salwane Smara region had revealed the presence of 27 compounds which presents 99.81 % as an essential oil total (TABLE 1).

The examination of these results shows that this essential oil is dominated by the existence of the iso-3-thujanol with 47.38 % percentage, followed by Santolina triene (11.67 %) and of camphor (10.95 %). We also note the presence of santolina alcohol (7.68 %), borneol (5.49 %), neo-iso-3-thujanol (3.74 %) and β-pinene (2.98 %).

The essence chemical profile of our *C. cinerea* from Smara is different from that of other regions. In fact, the essential oils of this plant of the Zagora's region (south Morocco) are composed majorly by the trans-thujone

TABLE 1 : Chemical composition of essential oil *Cotula cinerea* from Morocco

N°	KI	Compounds	%
1	911	santolina triene	11,67
2	932	β-pinene	0,16
3	940	camphene	1,97
4	955	thuja-2,4(10)-diene	0,61
5	979	β-pinene	2,98
6	983	cis-pinane	0,27
7	993	myrcene	0,37
8	1038	santolina alcohol	7,68
9	1064	p-mentha-3,8-diene	0,57
10	1074	camphenilone	0,09
11	1106	cis-thujone	0,43
12	1113	trans-thujone	0,53
13	1133	iso-3-thujanol	47,38
14	1141	amphre	10,95
15	1146	neo-iso-3-thujanol	3,74
16	1148	neo-3-thujanol	0,74
17	1156	iso-borneol	0,25
18	1164	borneol	5,49
19	1171	acetate de santolinyl	0,45
20	1176	iso-verbanol	0,6
21	1186	neo-iso-verbanol	1,13
22	1198	verbanol	0,96
23	1274	neo-3-acetate de thujanol	0,12
24	1290	trans-acétate de verbenyl	0,09
25	1294	3-acétate du thujanol	0,04
26	1406	sesquithujene	0,16
27	1460	dehydro-aromadendrane	0,38
Total			99,81

KI: Kováts Indices

(41.40 %), the acetate of cis-verbenyl (24.70 %), the 1,8-cineol (2.80 %), the santolina triene (7.2 %) and the camphor (5.5 %)[17].

Moreover, the essential oils analyses of *Cotula cinerea*, in the flowering period, coming from Oued Souf (South-eastern of Algeria) had revealed the presence of the majority components Δ-3-carene (30.73 %), thujone (21.73), santolina triene (18.58 %) and camphor (6.21 %)[12].

We notice that our essential oil is distinguished by the dominance of the 3-iso-thujanol while that of Algeria is co-dominated by Δ-3-carene and the thujone; Whereas, Santolina triene and camphor are present in the essential oils of the both countries with close percentages.

It is noted that thujone major component of Zagora (Southern Morocco) *Cotula cinerea* essential oils and Oued Souf (South East of Algeria) is totally absent in the essence of this species derived from Smara (Southern Morocco). In this latter, this component is replaced by a thujone derivative which is the iso-3-thujanol. Indeed, the high rate of this latter component detected for the first time in this species confirms that there exist several chemical races of Moroccan Sahara's aromatic and medicinal plant.

Our results agree with those of other authors[20,25,16] who concluded that *Cotula cinerea* essential oil is characterized by a chemiotype change which is due to several factors, such as : the plant's phenological stage, the genetic factors, the environment and the soil's nature.

Antibacterial and antifungal activity

The *C. cinerea* essential oils antibacterial and antifungal activity results are consigned in TABLE 2.

The four bacterial strains manifested the same sensitivity vis-à-vis to the *Cotula cinerea* essential oils. They were all inhibited at 1/500 v/v concentration. As

TABLE 2 : Antibacterial and Antifungal activity of *Cotula cinerea* essential oil from Morocco

Concentration v/v	1/100	1/250	1/500	1/1000	1/2000	1/3000	1/5000	T
Bacteria								
Escherichia coli	-	-	-	+	+	+	+	+
Bacillus subtilis	-	-	-	+	+	+	+	+
Staphylococcus aureus	-	-	-	+	+	+	+	+
Micrococcus luteus	-	-	-	+	+	+	+	+
Molds								
Penicillium digitatum	-	-	+	+	+	+	+	+
Penicillium expansum	-	-	+	+	+	+	+	+
Aspergillus niger	-	-	+	+	+	+	+	+
wood rot fungi								
Coriolus versicolor	-	-	-	+	+	+	+	+
Gloeophyllum trabeum	-	-	-	+	+	+	+	+
Coniophora puteana	-	-	-	-	+	+	+	+
Poria placenta	-	-	-	-	-	+	+	+

Note: (-), total inhibition; (+), growing

for mold, they were less sensitive than bacteria and their growth was stopped at 1/250 v/v concentration.

Concerning the wood rot fungi's four strains, we remark that *Poria placenta* manifest the greatest vulnerability towards this plant's essence with an inhibition threshold which reaches the low concentration 1/2000 v/v. The *Coniophora puteana* strain was also very sensitive to this essential oil with an inhibition rate of 1/1000 v/v. For the other two stem rot fungus *Coriolus versicolor* and *Gloeophyllum trabeum*, they were a little bit more resistant with an inhibitory concentration of 1/500 v/v.

Our results agree with those of El Bouzidi and al., (2012) which demonstrated that the essential oil of *Cotula cinerea* presented a great activity against all tested yeasts. These latters concluded that *Cotula cinerea* essence present a wide anticandidal activity spectrum[17].

Bensizerara and al.[10], found that n-butanol extract obtained from *C. cinerea* was very active against bacterial strains tested and *Candida albicans* fungus. The *Escherichia coli* strains and *Staphylococcus aureus* showed a high sensitivity to the essential oil of this plant, until the concentration 1/8 (v/v)[12].

The *C. cinerea* essential oils antibacterial and antifungal effectiveness is attributed to its chemical profile rich in monoterpenols. In fact, several previous studies have demonstrated the monoterpenic alcohols strong antimicrobial activity[5,7,26,33]. Previous works showed that a greater antimicrobial potential could be attributed to oxygenated terpenes in particular the phenolics compounds and terpenics alcohols[14,36].

In our study, the greatest antimicrobial power can be explained by the high content of iso-3-thujanol (47%). It is also noted that the essential oil of *C. cinerea* present a significant percentage of alcohol santolina. Indeed, Satrani and al.[35], demonstrated that alcohol santolina, main constituent of *Cladanthus mixtus* doted by a strong antibacterial and antifungal power.

The essential oil evaluated in this study contains a large variety of components which can be considered responsible for the observed antimicrobial activity. Although, the essential oil usually produced as a complex mixture, its bioactivity can be generally attributed to these majority monterpenics constituents. However, the synergetic action of the minority components cannot be ignored.

CONCLUSION

The *Cotula cinerea* essential oil average yield is 0.64 %. The essence of this plant is dominated by 3-iso-thujanol (47.38 %) which can be considered as a chemical marker of this species of Smara's (southern Morocco) origin.

In fact, the presence of this molecule with a high rate, detected for the first time in the *Cotula cinerea* essential oil, shows that it is characterized by a variability of chemical race. This divergence of the chemical profile is attributed to several factors, such as : the geographical origin, the inter and intraspecific variability genetic and the plant's phenologic stage.

furthermore, the *Cotula cinerea* essence showed a strong powerful antimicrobial against tested microorganisms. The wood's strains decay fungi were the most venerable of this plant's essence. This high bioactivity is due to its high content of terpenic alcohols, and more particularly its principles actives, the iso-3-thujanol and alcohol santolina. Indeed, the aromatic and medicinal plants extracts exploration, including essential oils showed that these ones were a potentially rich source of antimicrobial agent.

Finally, the promotion of human well-being deserves to combine the efforts in order to better promote our natural heritage of the Sahara, as well as the realization of further scientific research work on plants living in arid areas to exploit their pharmacological and therapeutic potentials.

REFERENCES

[1] R.P.Adams; Identification of Essential Oil Components by Gas chromatography/Mass Spectrometry, 4th Edition, Allured Publishing Corporation, Carol Stream, IL, **(2007)**.

[2] AFNOR; Huiles essentielles. Echantillonnage et méthodes d'analyse (Tome 1) – Monographies relatives aux huiles essentielles (Tome 2) mars, **1(2), (2000)**.

[3] M.S.B.Alaoui, B.Satrani, M.Ghanmi, A.Aafi, N.Amusant, S.El Antry, A.Chaoouch; Bioactivity and chemical quality of Ammodaucus leucotrichus ssp. leucotrichus Coss. & Durieu essential oils from

Morocco, Natural Products: An Indian Journal, **10(6)**, **(2014b)**.

[4] M.S.B.Alaoui, B.Satrani, M.Ghanmi, A.Aafi, A.Chaoouch; Effect of provenance on the chemical quality and bioactivity of essential oils from Moroccan fruits of Ammodaucus leucotrichus ssp. nanocarpus Beltrán, ScienceLib Editions Mersenne, **6**, 141003 **(2014a)**.

[5] F.Amarti, M.El Ajjouri, M.Ghanmi, B.Satrani, A.Aafi, A.Farah, A.Khia, A.Guedira, M.Rahouti, A.Chaouch; Composition chimique, activité antimicrobiennne et antioxydante de l'huile essentielle de *Thymus zygis* du Maroc, Phytothérapie, **9**, 149–157 **(2011)**.

[6] A.Ashnagar, N.Gharib, S.Foroozanfar; Isolation and identification of the major chemical components found in the upper parts of Teucrium Polium plants grown in Khuzestan province of Iran. Chinese Journal of Chemistry, **25**, 1171-1173 **(2007)**.

[7] H.K.Bencheqroun, M.Ghanmi, B.Satrani, A.Aafi, M.Aberchane, A.Echchelh, A.Chaouch; Effect of phenological stages on yield, chemical composition and bioactiviy of Artemisia mesatlantica essential oil of Morocco, Natural Products : An Indian Journal, **8(5)**, **(2012)**.

[8] B.Benjilali, S.Zrira; Plantes Aromatiques et médicinales atouts du secteur et exigences pour une valorisation durable. Actes éditions Institut agronomique et vétérinaire Hassan II Rabat Maroc, **(2005)**.

[9] A.Benseguni; Thèse de magister, Univ de Constantine, Tunisie, **(1989)**.

[10] D.Bensizerara, T.Menasria, M.Melouka, L.Cheriet, H.Chenchouni; Antimicrobial Activity of Xerophytic Plant (Cotula cinerea Delile) Extracts Against Some Pathogenic Bacteria and Fungi. Jordan Journal of Biological Sciences, **6(4)**, 266–271 **(2013)**.

[11] A.Chouikh, A.Chefrour; Seasonal biometric and dynamic monitoring of the Shihia plant Cotula cinerea Del (1831) and its accompanying plants in the Saharan region Oued-Souf (south-east of Algeria). International Journal of Science and Research (IJSR), **3(7)**, 826-833 **(2014)**.

[12] A.Chouikh, B.Mayache, M.C.Maazi, Y.Hadef, A.Chefrour; Chemical composition and antimicrobial activity of essential oils in Xerophytic plant Cotula cinerea Del (Asteraceae) during two stages of development: flowering and fruiting. Journal of Applied Pharmaceutical Science, **5(03)**, 029-034 **(2015)**.

[13] J.F.Clevenger; Apparatus for volatile oil determination: Description of New Type Clevenger. Am Perf & Ess Oil Review, 467-503 **(1928)**.

[14] M.Conladis, O.Tzakou, S.Kujundzić, M.Soković, N.Mimica-Dukić; Chemical analysis and antifungal activity of Thymus striatus. Phytotherapy Research, **18**, 40-42 **(2004)**.

[15] H.Dendougui, S.Seghir, M.Jay, F.Benayache, S.Benayache; Flavonoides from Cotula cinerea Del. Inst. J. Arom. Plants, **2(4)**, 589-595 **(2012)**.

[16] F.Dupont, J.L.Guignard; Abrèges botanique systématique moléculaire. 14ème édition révisée, Masson, **(2007)**.

[17] L.El Bouzidi, A.Abbad, K.Fattarsi, L.Hassani, D.Leach, M.Markouk; Chemical composition and anticandidal propréties of the essential oil isolated from aerial parts of Cotula cinerea : a rare and threatened medicinal plant in Morocco. Nat Prod Commun, 6 (10) pp 1491-1494 **(2011)**.

[18] S.Fellah, M.Ramdhane, M.Abderraba; Extraction et étude des huiles essentielles de la Salvia officinalis L. cueillie dans deux régions différentes de la Tunisie. J Soc Alger Chim, 193-202 **(2006)**.

[19] V.Hammiche, K.Maiza; Traditional medicine in Central Sahara: Pharmacopoeia of Tassili N'ajjer. J. Ethnopharmacol, **105**, 358–367 **(2006)**.

[20] R.Hammoudi, M.Hadj Mahammed; Contribution à l'étude de la composition chimique des huiles essentielles de la plante *Teucrium polium* SSP. *Geryrii* (Lamiaceae). Annales des Sciences et Technologie, **2(1)**, 01-05 **(2010)**.

[21] H.M.Jilali, H.Sereshti; Determination of essential oil components of *Artemisia haussknechtii* Boiss. using simultaneous hydrodistillation-static headspace liquid phase microextraction-gas chromatography mass spectrometry. J of Chromatography A, **1160(1 & 2)**, 81-89 **(2007)**.

[22] F.B.H.Kether, M.A.Mahjoub, S.A.Mahjoub, K.Belhaj Salah, A.N.Helal, Z.Mighri; Chemical composition, in vitro antifungal and antioxidant activities of essential oil from Cotula coronopifolia L. growing in Tunisia. African Journal of Microbiology Research, **6(20)**, 4388-4395 **(2012)**.

[23] E.Kovàts; Gas chromatographic characterization of organic substances in the retention index system. Advances in Chromatography chap, **7**, 229-247 **(1965)**.

[24] M.Larhsini, M.Markouk, J.T.Jaouhari, K.Bekkouche, H.B.Lazrek, M.Jana; The antipyretic activity of some Moroccan medicinal

plants. Phytother Res, **1**, S97-8 **(2002)**.

[25] I.Lmachraa, R.Fdil, N.Fdil, A.Mouzdahir; Huile essentielle de Santolina africana (Jord. & Fourr.) du Maroc: Composition chimique et isolement des deux principaux constituants. J Mater Environ Sci, **5(1)**, 67-72 **(2014)**.

[26] N.Mansouri, B.Satrani, M.Ghanmi, L.El Ghadraoui, A.Aafi; Étude chimique et biologique des huiles essentielles de Juniperus phoenicea ssp. lycia et Juniperus phoenicea ssp.turbinata du Maroc, Biotechnol. Agron. Soc. Environ., **15(3)**, 415-424 **(2011)**.

[27] M.Markouk, K.Bekkouche, M.Larhsini, M.Bousaid, H.B.Lazrek, M.Jana; Evaluation of some Moroccan medicinal plant extracts for larvicidal activity. J. Ethnopharmacol, **73**, 293–297 **(2000)**.

[28] M.Markouk, A.Redwane, H.B.Lazrek, M.Jana, A.Benjama; Antibacterial activity of Cotula cinerea Extracts. Fitoterapia, **70**, 314-316 **(1999)**.

[29] M.D.Ould El Hadj, M.Hadj-Mahammed, H.Zabeirou; Iventaire et recherche de l'usage des plantes spontanées médicinales de la pharmacopée traditionnelle de la région d'Ouargla (Sahara septentrional Est Algérien). Annales de l'Institut National Agronomique El Harrach, **22(1-2)**, 97-123 **(2003)**.

[30] Pharmacopée européenne; Huiles essentielles. Aetherolea, **(01)**, 2098 **(2008)**.

[31] P.Quezel, S.Santa; Nouvelle flore de l'Algérie et des régions désertiques méridionales. Tome II, Ed. CNRS, Paris, **(1963)**.

[32] A.Remmal, T.Bouchikhi, T.Bouchikhi, K.Rhayour, M.Ettayibi; Impoved method for determination of antimicrobial activity of essential oils in agar medium. J. Ess.Oils.Res., **5(2)**, 179–84 **(1993)**.

[33] R.Rhafouri, B.Strani, T.Zair, M.Ghanmi, A.Aafi, M.El Omari, A.Bentayeb; Chemical composition, antibacterial and antifungal activities of the Cedrus atlantica (Endl.) Manettiex Carrière seeds essential oil, Mediterranean Journal of Chemistry, **3(5)**, 1027-1036 **(2014)**.

[34] B.Satrani, A.Farah, M.Fechtal; Composition chimique et activité antimicrobienne des huiles essentielles de Satureja calaminthe et Satureja alpine du Maroc. Ann. Fals. Exp. Chim., **94(956)**, 241–50 **(2001)**.

[35] B.Satrani, M.Ghanmi, A.Farah, A.Aafi, H.Fougrach, B.Bourkhiss, D.Bousta, M.Talbi; Composition chimique et activité antimicrobienne de l'huile essentielle de *Cladanthus mixtus*. Bull. Soc. Pharm. Bordeaux, **146**, 85-96 **(2007)**.

[36] M.Soković, L.J.L.D.Van Griensven; Antimicrobial activity of essential oils and their components against the three major pathogens of the cultivated button mushroom, Agaricus bisporus. European Journal of Plant Pathology, **116**, 211-224 **(2006)**.

[37] R.De.Visiani; Plantae quaedam Aegypti ac Nubiae enumerate atque illustratae. Minerva, BDR-BV001729692-4541, **(1836)**.