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EVALUATION OF ANTI-SPASMODIC ACTIVITY OF *CITRUS* ESSENCES HARVESTED LOCALLY IN CHLEF REGION (ALGERIA): *IN VIVO* STUDY

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ABSTRACT

Essences were extracted by cold expression methods from four varieties of *Citrus* harvested in Chlefregion: *C. Sinensis, C. paradisi, C. reticulata*et *C. aurantium*. The characterization of the essences was done by gaschromatography coupled to mass spectrometry (GC/ MS) in order to evaluate the quality and composition of the semolecularspecies.

The antispasmodic activity of these species has been tested with a dose of 3 to 4 ml / kg by oral route in mice (MORINI) after the pain caused by aceticacid. The results obtained were compared with those of standard treatment (Spasfon). The evaluation of the percentage of inhibition of pain indicated that the *Citrus* essence possessed an anti-spasmodic effects with 74, 09±4, 64% for *Citrus aurantium,* 70,39±5,06% for *Citrus sinensis,* 64,12±4,45 % for *Citrus reticulate* and 59, 28±3,64 % for *Citrus paradisii.* We can conclude that *Citrus* essences significantly reduced spasm. This effect could beattributed to the bioactive compounds present in the essences such as: limonene.

KEYWORDS: Antispasmodic Activity, *Citrus*, Cold Expression, GC / MS

INTRODUCTION

In an attempt to find new cures for currentills, the scientific community has recently turned to the constituents of essential oils, for a significant number of volatile compounds. Thus, among the giftedbotanical families healing properties wefound: the Rutaceae which encompasses a wide range of aromatic plants, mainly located in tropical regions (Parray and *al*., 2012).

Citrus essences extracted by cold expression containnatural active compounds which are used in the pharmaceutical and biologicalfield: antimicrobial activity, antioxidant, anti-inflammatory, antispasmodic.....etc. (Watson, 2011).

The *Citrus* genusischaracterized by the presence of many bioactive secondary metabolism like: flavonoids (Tripoli and *al*., 2007), limonoid (Mamers, 2007) coumarins, sterols (Ladaniya 2008) volatileoil and alkaloids (He and *al*., 2010).

MATERIALS AND METHODS

Plant Material and Extraction Procedure

The four varieties of *Citrus*: *C. Sinensis, C. paradisi, C. reticulata*et *C. aurantium*, were collected in the Chlef

region in late February 2013. First the *Citrus* fruits were weighted, cleaned and peeled zest to recover. The extraction of essences is made by cold expression method.

Analysis of *Citrus* Essence by GC / MS

GC: Hewlett Packard Agilent 6890 N controlled by ChemStation (NIST98).

The chromatography Conditions are as follows

- Injection of 0.5µl Split mode 1/50
- Injector temperature: 250 ° C
- CapillaryColumn HP5MS (30 mx 0.25 mm x 0.25µm)
- Programming temperature: 35 ° C for 10 min; 4 ° C / min up to 250 ° C for 10 min.
- Flow of carrier gas: Helium (1ml/min)
- Mass spectrum: model Agilent 5973
- Temperatures: interface (280 ° C), source (230 ° C), quadrupole (150 ° C)
- The ionizationenergy of 70 eV.

To assess the quality and molecular composition of *C. sinensis* and *C. aurantium* essence, qualitative and quantitative analysis by GC / MS were performed.

ANIMALS

Male adult Swissmice weighing between 25 g and 30 g were used in this experimentation (The animals were provided by SAIDAL, MEDIA, Algeria). The animals were housed in cage and kept in a room temperature (22 ± 2°C) and lighting (light/dark cycle of 12 h, lights on at 7 am), withfood and water ad libitum. All experimental protocols were developed in accordance with the principles of ethics and animal welfarere commended. Each lot included 06 mice.

Study of Antispasmodic Activity by Writhing Test

The methoduse dissimilar to that described by Koster and *al*. (1959) and amended by Collier and *al*. (1968). Weinvestigated the antispasmodic activity in mice.

Principe of the Method

A pain reaction in micewasinduced by intraperitoneal injection of aceticacid in an amount of 0.10 ml / 10 g body weight (b.w). Pain manifested by a stretching of the hind legs and twists of the dorsal abdominal muscles (spasms), which canbe reduced by an antispasmodicproduct.

Procedure

The essences of the twovarieties are administered to micedivided into 4 lots. Each lot consists of 6 mouse strain NMRI, weight 25 ± 2 g.

- Lot (1): control group received the positive Spasfondilutedin 0.9% of physiological solution at dose of 200 mg / kg.
- Lot (2) At T0 received 0, 5 ml of *Citrus* essences by injection intraperitoneally.
- Lot (3): After 30 minutes, all micereceived 0.2 ml of solution of aceticacidat 1% by intraperitoneal route,

- Lot (4): After 5 min; counting the number of spasms which were observed directly in the mice for 10 min.

Calculating the Percentage of Reduction of Muscle Spasms

The percentage of reduction of muscle spasms (percent protection), is calculated using the formula according (Beloued 2005).

Statistical Analysis

Statistical analysis of the results was carried out by comparing each treated group to the control mices. The comparison was done by using Ki-Two test. A significant difference is represented by a $p < 0.05$; $n = 5$, represents the number of experiments per group.

RESULTS

Analyze of *Citrus* Essence Composition by CG/SM

Chemical analysis showed a determined number of components for bothspecies: 30 compounds for the essence of *C. aurantium* (99.92%) (Table 1), 17 compounds for the essence of *C. sinensis* (99.55%) (Table 2), 08 compounds for the essence of *C. reticulate* (99.92 %) (Table 3), and 31compounds for the essence of *C. paradise* (99.55%) (Table 4).

This analysis showed that these essences were constituted from a major component which is the "limonene" with different percentages (87.38% for *C. aurantium* essence, 86.29% for the essence of *C. sinensis*, 94,75% for the essence of *C. reticulate* and 82,98% for the essence of *C. paradisi*). Moreoverminor compound in essence of *C. aurantium* has minor compounds: β-pinene (3.59%), α-pinene (1.47%) and Furrancarboxaldehyde (1.13%), and in traces: phellandene (0.31%) Cyclohexane and (0.7%) (Table 1). In addition to limonene essence of *C. sinensis* are represented by β-pinene (2.33%), bicycloheptene (2.43%), aceticacid (2.94%), and in trace α-pinene (0.75%) and Octanol (0.16%) (Table 2). Moreover minor compounds essence of *C. reticulate* are represented by β - pinene(2.44%) ,1-5 dimethyl 1venyl (0.72 %), α pinène (0.91 %), β-phéllandrene (0.61 %) (Table 3). In addition to limonene, the essence of *C. paradise* has minor compounds: β-myrcene (2.66%), 2H-1-benzopyranone (2.07 %) and in trace:α-pinène (0.31 %) etl'acide n-hexadecanoique (0.88 %) (Table 4).

Table 1: Components (%) of *C. Aurantium* Essence Analyzed by GC /MS

Pics Number	Retention Time (min)	Chemical Composition	% Relative	Reconnaissance Level
1	17,85	**α-pinène**	**1,47**	**95**
2	20,03	β-phellandrene	0,31	91
3	21,04	**β-pinène**	**3,59**	**94**
4	23,52	**D-limonène**	**86,29**	**94**
5	23,83	1, 3, 7-octatriene	0,36	95
6	24,84	Formicacid	0,19	91
7	26,05	1,6-octadien-3-ol	0,83	94
8	29,81	α-terpineol	0,10	91
9	30,24	Decanal	0,20	91
10	31,81	β-myrcene	0,26	90
11	34,84	Cyclohexene	0,07	95
12	36,23	2,6-octadien-1-ol	0,15	91
13	37,68	Caryophyllene	0,14	99
14	39,63	Germacrene D	0,24	96
15	41,99	1,6, 10-dodecatrien-3-ol,	0,33	91
16	48,83	2(3H)-Naphthalenone	0, 55	99

Table 1: Contd.,

17	52,81	n-Hexadecanoicacid	0,78	99
18	54,87	7H-Furo(3, 2-g) (1) benzopyran-7-ol	0,28	93
19	56,59	Osthole	0,67	96
20	56,83	9,12-octadecadienoic acid	0,78	99
21	56,94	(z) 6, (z) 9-pentadecadien-1-ol	0,36	95
22	57,04	9-octadecenoic acid,	0,15	90
23	58,20	Cobalt	0,08	50
24	58,69	**2-Furancarboxaldehyde**	**1,13**	**49**
25	58,85	N.I.	0,16	35
26	59,64	Auraptenol	0,10	72
27	63,23	N.I.	0,07	27
28	65,03	1H-indole, 5-methyl-2-phenyl	0,21	62
29	65,11	Bis (2-ethylhexyl) phthalate	0,15	90
Total	/	/	**99.92**	/

Table 2: Chemical Composition (%) of *C. Sinensis* Essence Analyzed by GC/SM

Pics Number	Retention Time (min)	Chemical Composition	% Relative	Reconnaissance Level
1	2.50	Hexane	0, 11	90
2	5.99	**Aceticacid**	**2,94**	**91**
3	7.65	2-Butanone	1,24	86
4	17.82	α-pinéne	0,75	97
5	20.02	**Bicyclo (3, 1,1) heptane**	**2,43**	**91**
6	21.01	**β-pinène**	**2,33**	**91**
7	23.21	**Limonène**	**87,38**	**93**
8	24.81	1-Octanol	0.16	90
9	26.03	1-6-octadien-3-ol	0.81	91
10	29.50	4H-pyran-4one	1.35	81
11	30.24	Decanal	0.40	91
12	30.97	2-Furancarboxaldehyde	0.72	93
13	33.94	2-methoxy-4-vinylphenol	0.19	91
14	35.22	1,2-Cyclohexanediol	0.44	53
15	39.98	Naphthalene	0.25	91
16	52.70	n-Hexadecanoicacid	0.28	98
17	56.69	9,12-octadecadienoic acid	0.20	99
Total	/	/	**99.55**	/

Table 3: Chemical Composition (%) of *C. Reticulata* Essence Analyzed by GC/SM

Nombre de Pics	Temps de Rétention (min)	Composition Chimique	% Relative	Taux de Reconnaissance
1	1.727	Acide formique	0.24	4
2	2.012	Ether éthyle	0.13	91
3	17.907	α pinène	0.70	97
4	20.099	β-phéllandrene	0.61	91
5	21.079	β-pinene	2.44	94
6	23.169	**D-limonene**	94.75	94
7	26.086	1-5 dimethyl 1venyl	0.72	49
8	40.103	Naphtalene	0.42	99

Table 4: Chemical Composition (%) of *C. Paradisii*essence Analyzed by GC/SM

Nombre de Pics	Temps de Rétention (min)	Composition Chimique	% Relative	Taux de Reconnaissance
1	1.870	Ethanol	0.61	90
2	17.937	α pinene	0.91	96
3	20.116	β-phellandrene	0.49	90
4	21.114	β-myrcene	2.66	86
5	23.443	**Limonene**	82.98	93
6	23.840	Octariene	0.38	96
7	24.898	Acide formique	0.20	91
8	26.103	Octadien-3-ol	0.19	72
9	29.905	Cyclohexane	0.10	72
10	30.315	Decanal	0.29	86
11	36.331	α-cubebene	0.53	96
12	36.723	1,6cyclodecadiene	0.42	95
13	37,804	Caryophullene	1.25	99
14	38.945	Cycloundecatriene	0.17	98
15	39.111	Cycloheptasiloxane	0.08	90
16	39.741	1,6cyclodecadiene	0.31	96
17	40.863	Naphthalene	0.56	94
18	43.958	Sylane	0.19	55
19	48.139	Cyclononasiloxane	0.10	55
20	48.947	Naphthalenone	0.82	95
21	51.863	Cycloheptasiloxane	0.09	52
22	52.927	Acide n-hexadecanoique	0.88	98
23	55.267	Acide benzeneacetique	0.09	50
24	55.962	Acide 9,12octadecadienoique	0.09	99
25	56.674	Osthole	0.21	99
26	58.361	7 chloro-10-ethyl	0.17	46
27	58.741	2-naphthaldehyde	0.32	50
28	63.933	Cyclononasiloxane	0.16	52
29	65.263	Acide 1-2 benzenedicarboxylique	0.27	80
30	67.003	Acide benzenesulforique	0.22	56
31	67.538	2H-1-benzopyranone	2.07	46

Evaluation of Antispasmodic Activity

The results of our experiment showed that the number of spasmsde creased after treatment with essence *Citrus* (Table 5). Hence the evaluation of inhibition percentage of pain showed that *Citrus* essence possessed an anti-spasmodic effects with 74,09±4,64% for *Citrus aurantium,* 70,39±5,06% for *Citrus sinensis,* 64,12±4,45 % for *Citrus reticulate* and 59,28±3,64 % for *Citrus paradisii.*

Table 5: Percentage of Spasmsreduction after Administration of *Citrus* Essences

Lot	Number of Spasms	Percentage of Reduction
Négative control	70,25 ±0,51	0.00
Positive control	23,60±0,45	66,40±3,80
C. aurantium	18,20±0,73	74,09±4,64
C. sinensis	20,80±0,80	70,39±5,06
C. reticulata	25,20±0,71	64,12±4,45
C. paradisii	28,60±0,40	59,28±3,64

DISCUSSIONS

Analyze of *Citrus* Essence Composition by CG/SM

By studying the chemical composition of essential oils of *C. sinensis*, Moufida and Marzouk (2003) confirmed that these essential oils consistmainly of limonene. This compound varies between 68% and 98%, hence α-pineneis presentedonly in lowlevels (0.2% and 10.23%).

It is noted from this analysis that the acyclic compounds such as nerol and geraniol are absent in the species *C. aurantium* and C. *sinensis*. Gancel et al (2005) found the presence of these two compounds only in the essence of *C. limonum*. Several studies (Moufida and Marzouk, 2003; Belletiand *al*., 2004; Rehman and *al*., 2004) showed that generally Citrus essential oil was consistingmainly of monoterpene compounds (97%). Wher easother compounds, such as alcohols, aldehydes and esters are represented withlow contents of from 1.8 to 2.2%. Nogata and *al*., 2006, contested that flavonoids found in *Citrus* oils represented the non-volatile portion.

Evaluation of Antispasmodic Activity

Deraedt et *al*., 1980 through their study have demonstrated an increased proportions of prostaglandins PGE2α et PGEα in rats after injection with aceticacid. HenceDurate et *al.*, 1988 ; Hokanson et *al*.,1978 and Neo et *al*.,2005 have observed in the same condition, the release of mediators in the sympathicnervous system .

CONCLUSIONS

The results of the molecular composition of *Citrus* essential oils showed that the major component is "Limonene" with different percentages (87.38 % for *C. aurantium*, 86.29% for *C. sinensis*, 94,75% for the essence of *C. reticulate* and 82,98% for the essence of *C.paradisi*).

The evaluation of the percentager eduction in spasms showed that the essence of *Citrus aurantium* presented a signifi cant antispasmo diceffect of 74.09% and 70.39% for *Citrus sinensis*, 64,12±4,45 % for *Citrus reticulate* and 59, 28±3,64 % for *Citrus paradisii*..The limonene could be the component which is responsible for this activity.

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