International Journal of Applied and Natural Sciences (IJANS)
ISSN(P): 2319-4014; ISSN(E): 2319-4022
Vol. 4, Issue 5, Aug - Sep 2015, 21-30

International Academy of Science, Engineering and Technology
Connecting Researchers; Nurturing Innovations

ANTI-INFLAMMATORY ACTIVITY OF *CITRU S*ESSENCES HARVESTED LOCALLY IN CHLEF REGION (ALGERIA): *IN VIVO* STUDY

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ABSTRACT

Essence was extracted by cold expression methods from four varieties of *Citrus* harvested in Chlef region: *C. Sinensis, C. paradisi, C. reticulate* and *C. aurantium*. The characterization of the essence was done by gas chromatography coupled to mass spectrometry (GC/ MS) in order to evaluate the quality and composition of these molecular species.

The anti-inflammatory activity of Citrus Essence was tested with a dose of 3 and 4 ml / kg by intra- peritoneal route after induced the paw edema by carrageen in in mouse model (MORINI), the results obtained were compared with those of the standard treatment.

The results of our experiment showed that Citrus essence had a significant anti-inflammatory effect via evaluation of the percentage of inhibition of edema and after a period of 120 min with *C.reticulata* and *C. aurantium*, hence for *C.paradise* and *C. Sinensis* were after 150 min. In conclusion we find that essence extracted from *Citrus* species reduces with significant manner the edema, whereas the molecule responsible for this effect could be limonene.

KEYWORDS: *Citrus,* Cold Expression, Anti-Inflammatory Activity, GC / MS

INTRODUCTION

Inflammation is a reaction of defense of the body against various threats and attacks that can be physical, chemical or biological. the current treatment of the inflammations uses anti-inflammatory drugs ((glucocorticoids) and non steroidiananti-inflammatory (NSAIDs) (Gaziano and *al*., 2006). In developing countries, plants possessing anti -inflammatory activity could include a therapeutic alternative in the anti - inflammatory treatment (Khalil and *al*., 2006)

The biological activity of many botanical extracts has been widely studied, but little research has been established to evaluate the anti -inflammatory activity of essences extracted from the peel of *Citrus*. In traditional medicine, the fruit of Citrus is used as antipyritique, anti - inflammatory, anti -toxic and sedative (Arias and Romoin - Laca, 2005). The *Citrus* genus is characterized by the presence of many bioactive secondary metabolism: flavonoids (Tripoli and *al*., 2007.), Limonoid (Mamers, 2007) coumarins, sterols (Ladaniya 2008) volatile oil and alkaloids (He and *al*., 2010). The aim of the study was to evaluated the anti-inflammatory activity of the essence extracted from *Citrus* .

IMATERIALS AND METHODS

Plant Material and Extraction Procedure

The four varieties of *Citrus*: *C. Sinensis, C. paradisi, C. reticulate* and *C. aurantium*, were collected in the Chlefregion 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 Agilent6890N controlled by ChemStation (NIST98).

The chromatography conditions are as follows:

- Injection of 0.5µl Split mode 1/50
- Injectort emperature: 250 ° C
- Capillary Column 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.

Determination of Anti- Inflammatory Activity

The reaction of inflammation was induced by the injection of carrageenan in the hind paw plantar of mice causing the occurrence of swelling of the metatarsal region (Winter et al., 1963). Then the animals were divided 03 groups as follows:

***Negative Control Group*:** An aqueous solution of 1% carrageenan was injected in intra- peritoneal (IP) to mice.

***Positive Control Group*:** An aqueous solution of 1% carrageenan was injected IP in the mice in addition treatment with declophenac.

Treated Group: An aqueous solution of 1% carrageenan was injected in IP to mice in addition to treatment with 3

and 4 ml / kg of Essences of *C. Sinensis, C. paradisi, C. reticulata*and *C. aurantium.*

Evaluation of the Anti- Inflammatory Activity

The anti-inflammatory activity was evaluated by calculating the percentage of inhibition of edema (% INH). The average of treatments groups with essence of *Citrus* or declophenac were compared to those of the control group treated with saline solution only. The percentage inhibition of inflammation is calculated using the formula (Sango and *al*., 2006):

$$\%\ Inhibition\ = \frac{final\ paw\ volume\ of\ control\ group - final\ paw\ volume\ of\ treated\ group}{final\ paw\ volume\ of\ control\ group} \times 100$$

Statistica Analysis

Statistical analysis of the results was carried out by comparing eachtreated 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 both species: 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. reticulata*(99.92 %) (Table 3), and 31compounds for the essence of *C. paradisi*(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 %) (Table3). 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 %) (Table4).

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
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

The Evaluation of the Anti- Inflammatory Activity

The results showed that the Citrus essence reduced with a significant manner the volume of the edema. Results obtained were compared with those of sodium declophenac, and those of the negative control (Table5).

After intra- peritoneal (IP) administration of distilled water, carrageenan increases the diameter of the paw pads : 15.57% ± 0.09, 17.74 ± 0.11 %, 28.13 % ± 0.12, 21.99 ± 0.08, 32.26 ± 0.33 %, 45.38 % ± 0.12 and 55.16 ± 0.09 %, respectively ; at 30min, 60min, 90min, 120min, 150 min, 180 min and 210 min. The IP administration of 0.5 ml of declophenac significantly prevent the increase of the anti-inflammatory activity : 46.42 ± 1.7, 56.63 ± 4.2, 74.51 ± 0.8, 79.09 ± 0.6, 89.08 ± 1.6 and 100 at 30min, 60min, 90min, 120min, 150 min to 180 min respectively after administration of carrageenan (Table 3). These results are significantly different from the negative control.

The IP administration of *Citrusreticulata* essence with 1 % of the dose prevent significant swelling of the paw of mice after 30 min, 60 min, 90 min up to 120 min with percentages of increases in the respective anti -inflammatory activity of 29,28± 1.4, 66.36±3.1, 87.58 ±.0.4, 98.49± 1.9, 100% (Table 5).

The IP administration of *Citrus aurantium* essence with 1 % of the dose prevent significant swelling of the paw of mice after 30 min, 60 min, 90 min up to 120 min with percentages of increases in the respective anti -inflammatory activity of 39.39 ± 1.3, 52.67 ± 1.9, 67.7 ± 2.0, 90.27 ± 1.9, 100% (Table 3).

The IP administration of *Citrus sinensis* essence at a dose of 1 % significantly prevents the paw edema of mouse after 30 min, 60 min, 90 min, 120 min up to 150 min with percentages increases the respective anti- inflammatory activity of 34.54 ± 1.7, 69.19 ± 2.1, 65.1 ± 0.8 76.7 ± 1.4, 96.17 ± 0.7 and 100% (Table 5).

The IP administration of *Citrus paradisi* essence at a dose of 1 % significantly prevents the paw edema of mouse after 30 min, 60 min, 90 min, 120 min up to 150 min with percentages increases the respective anti- inflammatory activity of 24.54± 1.6, 59.1 ±1.3, 62.1±1,3, 76.7± 1.1, 96.17 ± 1.1. and 100% (Table 5).

Edema caused by carrageenan in the paw of the mouse has three distinct phases: a first phase that involves histamine and 5-hydroxytryptamine that promote vasodilation, plasma exudation and edema; a second phase that uses the kinins as mediators increase vascular permeability and a third phase which is supposed to be the mediator prostaglandin associated with leukocyte migration into the inflamed area (Lindsey and *al*., 1999. Bouhassira and Attal, 2000).

The inflammatory response include the recruitment of leukocytes and the release of inflammatory cytokines such as : TNF - α, IL- 6, IL- 10. Indeed, many essential oils showed inhibitory activity against the production of cytokines (Kumer and *al*., 2013). Kundsen and *al*., 2011 have shown that flavonoids and limonoid present in the plant Citrus are responsible for the anti - tumor activity and anti-inflammatory *in vitro* and *in vivo*study .

Citrus species are rich in terpene (sesquiterpene lactone aldehyde) and oxygenated compounds (ketones)that are probably responsible for this activity (Descheaeeaker, 2003). The essential oil of *Citrus* seems to have an anti - inflammatory activity via inhibition of NO production in target areas (Yang and *al*., 2009). This effect is mainly due to the presence of limonene, the major component of citrus peel.

Table 5: Percentage Inhibition of Edema in Function of Time for Tow Variety of Citrus (C. *Reticulata* and *C. Paradisi)*

Temps	During Injection	30 min	60 min	90 min	120 min	150 min	180 min	210 min
Control(-): distilled water	15,53±0,15	15,57±0,09	17,74±0,11	28,13±0,12	21,99±0,08	32,26±0,33	45,38±0,12	55,16±0,09
Control (+): declophenac	43,47±1,5	46,63±0,8	46,42±1,7	56,63±4,2	74,51±0,8	79,09±0,6	89,08±1,6	100
Citrus paradisi	15,21%±1,5	24,54%±1,6	59,19%±1,7	62,1%±1,3	76,7%±1,1	96,17%±1,1	100%	100
Citrus reticulata	17,87%±5,1	29,28%±1,4	66,36%±3,1	87,58%±0,4	98,49%±0,6	100%	100	100
Citrus aurantium	15,21±1,8	39,39±1,3	52,67±1,9	67,7±2,0	90,27±1,9	100	100	100
Citrus sinensis	45,21±0,8	34,54±1,7	69,19±2,1	65,1±0,8	76,7±1,4	96,17±0,7	100	100

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 thatthese essential oils consistmainly of limonene. This compound varies between 68% and 98%, hence α-pineneis presented only 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; Belleti and *al*., 2004;. Rehman and *al*., 2004) showed that generally Citrus essential oil was consisting mainly of monoterpene compounds (97%). Where as other 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.

The Evaluation of the Anti- Inflammatory Activity

Edema caused by carrageenan in the paw of the mouse has three distinct phases: a first phase that involves histamine and 5-hydroxytryptamine that promote vasodilation, plasma exudation and edema; a second phase that uses the kinins as mediators increase vascular permeability and a third phase which is supposed to be the mediator prostaglandin associated with leukocyte migration into the inflamed area (Lindsey and *al*., 1999. Bouhassira and Attal, 2000).

The inflammatory response include the recruitment of leukocytes and the release of inflammatory cytokines such as: TNF - α, IL- 6, IL- 10. Indeed, many essential oils showed inhibitory activity against the production of cytokines (Kumer and *al*., 2013). Kundsen and *al*., 2011 have shown that flavonoids and limonoid present in the plant Citrus are responsible for the anti - tumor activity and anti-inflammatory *in vitro* and *in vivo*study .

Citrus species are rich in terpene (sesquiterpene lactone aldehyde) and oxygenated compounds (ketones) that are probably responsible for this activity (Descheaeeaker, 2003). The essential oil of *Citrus* seems to have an anti - inflammatory activity via inhibition of NO production in target areas (Yang and *al*., 2009). This effect is mainly due to the presence of limonene, the major component of citrus peel.

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*).

Evaluation of percentage of inhibition indicated that the essence of *Citrus aurantium* and *C.reticulata* has a significant anti-inflammatory effect after a period of 120 min and after 150 min for *Citrus sinensis* and *C. paradisi*. Limonene may The limonene could be the component which is responsible for this activity.

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