

Anti-inflammatory Activity Citrus Essence (*C. Sinensis* and *C. aurantium*) Local to Chlef Region (Algeria): *In vivo* study

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Abstract

Essence was extracted by cold expression methods from two varieties of Citrus harvested in Chlef region: *C. sinensis* 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 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. aurantium*, hence for *C. sinensis* was 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: *C. sinensis*; *C. aurantium*; 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-steroidal anti-inflammatory (NSAIDs) like aspirin which have a number of sides effect that can be injuries after a long term of use [1]. In developing countries, plants possessing anti-inflammatory activity could include a therapeutic alternative in the anti-inflammatory treatment [2].

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 antipyretic, anti-inflammatory, anti-toxic and sedative [3]. The Citrus genus is characterized by the presence of many bioactive secondary metabolism: flavonoids [4], Limonoid [5] coumarins, sterols [6] volatile oil and alkaloids. The aim of the study was to evaluate the anti-inflammatory activity of the essence extracted from Citrus.

Material and Methods

Plant material and extraction procedure

The two varieties of Citrus: *C. sinensis* and *C. aurantium* were collected in the Chlef region in late February 2012. First the Citrus fruits are weighed, cleaned and peeled zest to recover. The extraction of essences is made by cold expression of zest.

Analysis of Citrus essence by GC / MS

Chromatographic conditions: GC: Hewlett Packard Agilent6890N controlled by ChemStation (NIST98). The chromatography conditions are as follows:

- Injection of 0.5µl Split mode 1/50
- Injector temperature: 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)

- The ionization energy of 70 eV.

Animals

Male adult Swiss mice 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), with food and water ad libitum. All experimental protocols were developed in accordance with the principles of ethics and animal welfare recommended

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 [7]. 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

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injected in IP to mice in addition to treatment with 3 and 4 ml / kg of Essences of *C. sinensis* 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

Statistical analysis

All values were expressed as mean ± standard error of mean (SEM). Comparing means for increasing the volume of the paw pads and edema inhibition percentage was made with the Chi- square test. A significant difference is represented by a p <0.05; n = 5, represents the number of experiments per group.

Results

Analysis of Citrus essence by GC /MS

Chemical analysis revealed a certain number of components for

both species: 30 compounds for the essence of *C. aurantium* (99.92 %) (Table 1), and 17 compounds for the essence of *C. sinensis* (99.55 %) (Table 2).

This analysis showed that these species have a majority compound which is limonene with different percentages (87.38 % for the essence of *C. aurantium* and 86.29 % for the essence of *C. sinensis*). Moreover minor compounds essence of *C. sinensis* are represented by β - pinene (2.33 %), bicycloheptene (2.43 %), acetic acid (2.94 %), and in trace α - pinene (0.75 %), and octanol (0.16 %) (Table 2) .

In addition to limonene, the essence of *C. aurantium* has minor compounds : β -pinene (3.59 %), α - pinene (1.47 %) and Furrancarboxaldehyde (1.13 %) and in trace : phellandene (0.31 %) Cyclohexane (0.7 %) (Table 1). By studying the chemical composition of essence of *C. sinensis*, Moufida and Marzouk [8] confirmed that these essential oils are composed mainly of limonene. This compound varies

It is noted from this analysis that the acyclic compounds such as nerol and geraniol are absent in the *C. reticulate* and *C. paradise* [9] found the presence of these two compounds only in the essential oil of *C. limonum*. Several studies [8,10] showed that generally essential oil of Citrus are composed mainly of monoterpene compounds (97%).

Pics number	Retention time (min)	Chemical composition	% relative	Reconnaissan celevel
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 1: Components (%) of *C. aurantium* Essence analyzed by GC /MS

Pics number	Retention time (min)	Chemical composition	% relative	Reconnaissancelevel
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 -pinène	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 2: Components (%) of *C. sinensis* Essence analyzed by GC/SM

While other compounds such as alcohols, aldehydes and esters are represented with low percentage: 1.8 to 2.2 %. Nogata et al., 2006 found that flavonoids are found in citrus oils which represent the non-volatile portion.

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 (Table 3). After intra- peritoneal (IP) administration of distilled water, carrageenan increases the diameter of the paw pads : 15.57% \pm 0.09, 17.74 \pm 0.11 % , 28.13 % \pm 0.12, 21.99 \pm 0.08, 32.26 \pm 0.33 %,45.38 % \pm 0.12 and 55.16 \pm 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 \pm 1.7 , 56.63 \pm 4.2, 74.51 \pm 0.8, 79.09 \pm 0.6, 89.08 \pm 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 *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 \pm 1.3, 52.67 \pm 1.9, 67.7 \pm 2.0, 90.27 \pm 1.9, 100% (Table 3).

The IP administration of *Citrus sinensis* sence 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 \pm 1.7, 69.19 \pm 2.1, 65.1 \pm 0.8 76.7 \pm 1.4, 96.17 \pm 0.7 and 100% (Table 3).

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 [11].

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 [12,13] 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)

	During l'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
<i>Citrus aurantium</i>	15,21 ± 1,8	39,39 ± 1,3	52,67 ± 1,9	67,7 ± 2,0	90,27 ± 1,9	100	100	100
<i>Citrus sinensis</i>	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

Table 3: Percentage inhibition of edema in function of time for tow variety of Citrus(*C. aurantium* and *C. sinensis*).

and oxygenated compounds (ketones) that are probably responsible for this activity [14]. The essential oil of Citrus seems to have an anti-inflammatory activity via inhibition of NO production in target areas [15]. This effect is mainly due to the presence of limonene, the major component of citrus peel.

Conclusion

The results of the molecular composition of Citrus essential oils showed that the major component is the "Limonene" with different percentages (87.38 % in *C. aurantium* and 86.29% in *C. sinensis*). Evaluation of percentage of inhibition indicated that the essence of *Citrus aurantium* has a significant anti-inflammatory effect after a period of 120 min and after 150 min for *Citrus sinensis*. Limonene may be the molecule responsible for the anti-inflammatory activity.

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