Composition, Physicochemical Analysis, Antimicrobial and Anti- Inflammatory Activities of the Essential Oils obtained from *Ruta chalepensis. L* Growing Wild in Northen of Algeria

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Summary: *Ruta chalepensis* L is an aromatic plant belonging to Rutaceae family which is widely found in North Africa, particularly in Algeria. Our study has focused on extraction by steam distillation, physicochemical analysis, gas chromatography coupled with mass spectrometry analysis (GC-MS) and biological activity of essential oils (EOs) obtained from the aerial part of this plant, collected from the region of Hamman Melouane (Blida,Algeria).The results revealed that the extraction yield was 0.4 ± 0.03 % (w/w) and a total of seventeen compounds of essential oils were identified with 2-undecanone (35.51 %), 1- decanol-2 methyl (8.62 %) and 2-dodecanone (6.86 %) appeared as dominated compounds. Results showed also that our EO_S presented an adequate quality with the following parameters: acid index; 2.86, ester index; 22.44, refractive index; 1.43 and density of 0.84.In addition, our finding revealed that the extracted oils had exerted a variable antimicrobial activity. They presented a moderate inhibition zone with diameters of 25, 27 and 28 mm against *Klebsila pneumoniae* ATCC4352,*Saccharomyces cerevisiae* ATCC2601 and *Candida albicans* ATCC24433 respectively.The minimum inhibitory concentration (MIC) was further investigated and it has been observed that the lowest concentration of EOs was found to be 0.03 % (v/v) against *Candida albicans* ATCC24433 and *Saccharomyces cerevisiae* ATCC2601, contrary to *Klebsila pneumoniae* ATCC4352 which had an MIC value of 0.125 % (v/v). Moreover, the most sensitive microorganisms to oils were *Saccharomyces cerevisiae* ATCC2601 and *Bacillus subtilis* ATCC9372 with a minimal fungicidal concentration (MFC) and minimal bactericidal concentration (MBC) of 0.06 % (v/v) and 0.125 % (v/v), respectively. Furthermore, it had exhibited a high significant anti-inflammatory activity with edema reduction rate of 53.59 % ($p < 0.004$).Consequently, this study highlighted that the extracted EOs could be considered as potentials anti microbial and ant-inflammatory candidates in therapeutic and pharmaceutical applications.

Keywords: *Ruta chalepensis* L, Essential oils (EOs), Physicochemical analysis, GC-MS analysis, Antimicrobial activity, Anti-inflammatory activity.

Introduction

Inflammation is a physiological defense mechanism resulting from a body attack to isolate and repair the tissue damage. It plays a protective role by participating in the process of innate defense of the body and manifests itself clinically by four cardinal signs such as redness, heat, pain, and edema [1]**.** Its clinical treatment is currently dependent on non-steroidal or steroidal chemical therapeutics [2]**.** However, all these drugs carry potential toxic effects. Several authors in previous studies [3] have suggested that the risk of gastrointestinal bleeding was significantly associated only with acute use of non-steroidal anti-inflammatory drugs at regular dose of aspirin, diclofenac, ketorolac, naproxen or nimesulide whereas piroxicam increased the risk of bleeding in both acute and chronic therapy. To limit these disadvantages uses, several researchers have been interested to another alternative solution which consists to investigate the medicinal plants for obtaining natural substances with minimal side effects. These plants have long been used for treating a wide range of diseases, including inflammatory processes of diverse origins, and have provided symptomatic relief comparable to that obtained from allopathic medicines [4]**.** Moreover, they are considered as an important source for new active molecules for various uses such as antibacterial and antifungal activities towards some pathogenic microorganisms resistant to antibiotics and antifungal agents. In general, this microbial activity is a primary mode of deterioration of many foods and is often responsible for the loss of its quality and safety, causing thus important human diseases.

Among the countless species of medicinal interest, there are plants belonging to the Rutaceae family which was distributed in temperate and tropical countries and was introduced to America after the Spanish conquest [5]. One of the widely diffused species in the Mediterranean area is *Ruta chalepensis* commonly known as rue or fringed rue which is among the most-used in traditional medicine

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of Algeria and many other countries. This plant is used for the treatment of a variety of diseases since Antiquity [6]. It is described for its emmenagogue, antispasmodic rubfiant, and escharotic powders [7] and is often used for many others varied conditions as hysteria, epilepsy, vertigo, colic, intestinal worms, poisonings, headache, anxiety and eye problems [8]. The decoction of *Ruta chalepensis* is used as a medicinal remedy against evil eye and for "spiritual cleansings" [9] whereas the infusions of its fresh leaves are widely used as treatment for gastric disorders, headache and rheumatism, as well as for their diuretic, anti-inflammatory and anti-spasmodic properties [10]. This plant would be also a repellent for mosquitoes and snakes and an antidote for poisonings by the venoms of snakes and scorpions, per os and poultices at the bite or sting [11].Furthermore, it has been shown that the aqueous extract of the *Ruta chalepensis* leaves has a spermotrophic action demonstrated by the increase in sperm count, motility, living percent, and decrease in encountered sperm abnormalities. The hormonal profile is also influenced since the testosterone and follicle stimulating hormone levels are significantly increased with no change in the leutinizing hormone and prolactin levels [12]. On general, all its biological activities could probably be correlated with the presence of natural products such as alkaloids, coumarins, phenols, saponins, flavonoids , triterpens and essential oils. They are natural, volatile liquid, complex compounds characterized by a strong odor, rarely colored, soluble in lipid and organic solvents. It could be synthesized by all plant organs, *i.e*. buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood or bark, and are stored in secretary cells, cavities, canals, epidermic cells or glandular trichomes [13]. They are composed with terpenoids and their oxygenated derivatives which are known for their particular pharmacological properties such as antihelmintic, anti-inflammatory, emmenagogue, spasmolytic effects, as well as antibacterial and antifungal activities [14].

Our study is focused on ; (I) to extract essential oils and to determinate their physicochemical indexes and chemical composition using gas chromatography coupled with mass spectroscopy GC-MS,(II) to evaluate their biological activities mainly antimicrobial and anti-inflammatory properties.

Experimental

Biological material (Ruta chalepensis)

The plant was identified at the level of the National Institute of Agronomic Research (INRA). It was collected in May 2012, from the region of Hamam Melouane (Blida, Algeria) and dried further in shadow, at an airy place.

Essential oils extraction

Isolation of essential oils was performed by steam distillation of 300 g of dry aerials parts (Stems, leaves and flowers) for 4 hours. Every 15 minutes, the oils obtained were collected, dried over anhydrous sodium sulfate (Na_2SO_4) and further conserved at 4 °C in sealed opaque bottles. The essential oils yield (Y) is expressed in percentage (%) and calculated by the following formula:

$$Y(\%) = (w_{EO}/w_{PM}) \cdot 100$$

where ; w_{EO} and w_{PM} correspond respectively to the weight of the essential oil and the weight of the dried plant material in grams.

Animals

Albino mice weighting 19 to 21 g were used in this study. They were obtained from the laboratory of pharmacology and toxicology (research and development center, Algiers Algeria).These mice were housed in plastic cages with stainless steel grids (10 mice/cage) under controlled conditions at 50 % of humidity, a temperature of 20 to 24 °C and at a 12 hours of light/dark cycle. All experiments were in accordance with the guidelines for the care and use of laboratory animals published by the US National Institute of Health (NIH publication No 85–23, revised 1985).

Microorganisms

Five strains from the international collection (American type culture collection) were used for testing the antimicrobial activity. They named *Escherichia coli* ATCC 4157, *Klebsiella pneumoniae* ATCC 4352, *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 9372, *Candida albicans* ATCC 24433 and *Saccharomyces cerevisiae* ATCC2601. All these strains were maintained and tested separately in their specific medium. The bacterial strains were cultured overnight at 37°C on nutrient agar containing (g/l): glucose 20, peptone 5 and agar-agar 15, whereas the fungi strains were developed at 28 °C on Sabouraud Dextrose Agar (g/l): peptone 10, glucose 40 and agar-agar 15.

Physicochemical characterization of essential oils

The organoleptic properties such as appearance, color and odor have been done by

observing and directly inhaling the essential oils. The refractive index at room temperature was carried out using the ABBE refractometer while the density was estimated using densitometer. In addition, the miscibility to ethanol, acid and ester indexes were determined [15].

Essential oils analysis by GC / MS

The different constituents of essential oil were identified and quantified using HP 6890 gas chromatography coupled to HP5973 mass spectrometer. 0.1 µl of sample were injected with split mode (split ratio of 1:10) into a column capillary HP-5MS containing 5 % of phenyl and 95 % of methypolyciloxane (30 m x 0.2 mm with 0.25 µm of film thickness).The separation was carried out using helium as carrier gas with flow rate of 1.5 ml/min and oven temperature programmed from 60 °C to 220 °C for 10 minutes at a rate of 3 °C./min. Individual components were identified by comparing their mass spectra with those of authentic compounds previously analyzed and stored in the MS database (NIST 2002 and Wiley library) and also by comparing their GC-retention indices with those recorded in the literature data. The retention indices (RI) were determined for all the volatile constituents using retention times (RT) of an n-alkane homologous series, ranging from C_8 to C_{30}, analyzed in same conditions using a linear temperature programmed equation [10]. The quantification of essential oils composition is expressed on percentage and it was determined by integration of GC peak areas without using correction factors.

Antimicrobial activity evaluation

The qualitative evaluation of the antimicrobial activity for the essential oils obtained from *Ruta Chalepensis* was carried out using agar disc diffusion method ; whereas the quantitative evaluation was conducted using dilution method for determining the minimum inhibitory concentration (MIC) , and both minimum bactericidal and fungicidal concentrations (MBC and MFC).

Agar disc diffusion method

The culture media of Muller Hinton agar and Sabouraud were individually inoculated by 200 µl of fresh bacterial and fungal suspensions at concentrations of 10^7 to 10^8 CFU/ml. Then, 20 µl of essential oils were injected into sterile paper discs with 6 mm of diameter, which were deposited further separately on the surface of each inoculated agar plate and incubated at 37°C during 24 h and at 25 °C during 48 h for bacteria and fungi, respectively. After the incubation period, the antimicrobial activity was determined by measuring the diameters of the inhibition zone surrounding each disc. It was estimated as follows: Diameters greater than 20 mm (extremely sensitive strain); Diameters between 15 to 19 mm (very sensitive strain); Diameters between 9 to14 mm (sensitive strain); Diameters less than 8 mm (resistant strain) [17].

Minimum Inhibitory Concentration

From the stock solution of essential oil, serial dilutions were performed for obtaining concentrations in the range of 0.03 to 2 % (w/v).The culture media were inoculated separately by microbial suspension in the appropriate concentration and the plates, obtained with impregnated oils disc, were incubated at 37 °C during 24 h and at 25 °C during 48 h for bacteria and fungi respectively. The lowest concentrations of essential oils without visible growth were considered as MIC.

Minimum Bactericidal Concentration and Minimum Fungicidal Concentration determination

The MBC and MFC were evaluated for bacteria and fungi respectively. They were conducted with the same steps followed in MIC test except in this method the serial sub-culturing occurred for the oil concentrations that have no visible growth. The MBC and MFC defined as the lowest concentration of essential oil that destroys completely the initial inoculums.

Anti inflammatory activity evaluation

The anti-inflammatory activity was carried out using the carrageenan-induced paw oedema method [18]. Eighteen male mice were used in this experiments and they were divided into three groups of six animals each (n = 6).All animals were fasted 18 h before the test. The first group was considered as control and it was received orally 0.5 ml of physiological saline solution (0.9 % NaCl).The second group was injected with 0.5 ml of essential oils at the dose of 150 mg/kg, whereas the third one was treated with same volume of reference drug (dichlofenac 30 mg/kg).After thirty minutes of oral administration, paw oedema was induced by injecting 0.025 ml of carrageenan, dissolved in physiological saline solution (1 % p/v), into the plantar aponeurosis of the left hind paw whereas the right hind paw was taken as control. Then, the mice were sacrificed after four hours of the oral administration using ether and a 6 mm-diameter sections of the right and left ears

were cut and weighed. The percent oedema inhibition was calculated for each animal group using the following Formula;

Percentage inhibition = (PZ-PZo) control – (PZ-PZo) treated) / (PZ-PZo) control X 100, Where:

PZ is paw size after carrageenan injection, and PZo is paw size before carrageenan injection..

Statistical analysis

The statistical analyses were performed with SPSS 8 (SPSS Inc,USA) and the results were presented as the means ± standard deviation (SD) of three repeated estimations. All the data were statistically evaluated using analysis of variance (ANOVA) with Student's test and the significant difference between groups was set at $P < 0.05$.

Results and Discussion

Yield and kinetic extraction of essential oils

It has been calculated that the humidity level of plant in the fresh state is 68 % which is lower than that recorded in literatures [19], who has found a value of 77 % while working on the same species. This variability could be explained by the difference in climatic conditions, place and period of the harvest. In addition, the essential oils yield obtained from the tested plant is 0.40 ± 0.03 % (w/w) which is similar to that obtained by an Algerian author [19] who has found 0.41 % and more than that recorded by other authors [20] (0.12 %) , whereas it seems less than the value signaled in previous study [21] (0.84 %).This variation in yield can be attributed not only to the plant origin and extraction technique but also to the collection period of the plant material [22]. As mentioned in Fig. 1, the extraction of the EOs from the *Ruta chalepensis* is carried out during 4 hours. Its evolution is performed through two steps. The first one is characterized by a very slight increase during the first 30 min .It was followed further by another step which is well-known by a very significant increase in yield from 0.25 % to 0.40 % in range time (30 to 180 mn).After this duration, the evolution has been stopped.

Organoleptic and physicochemical analyses of essential oils

The essential oils appear liquid, mobile, and clear with brownish yellow color and pleasant odor. Additionally, the physicochemical properties (Table-1) such as: refractive index, acid index, ester index, ethanol miscibility, and density at 20 °C, constitute main factors for quality control of the EOs. They indicated that our findings are in agreement with those cited by literatures [21] while they are lower comparing to those obtained by an Algerian author [19] who has found the following results; density of 0.929, ester index 50.36 and acid index 8.62.In addition, our oils quality is different to the Ethiopian oils quality obtained from *Ruta chalepensis* wich are characterized by density value of 0.725 and acid index value of 6.73 [23]. This variability could be related to the harvest region. In general, these parameters are not sufficient. It is therefore necessary to supplement them with GC/MS analysis for identification of its chemical composition [24].

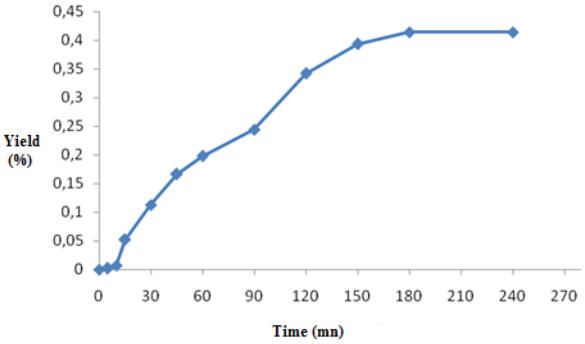

Fig. 1: Evolution of the essential oil obtained from *Ruta chalepensis* as function as the extraction time.

Table-1: Physicochemical characterization of the essential oils of *Ruta chalepensis.*

Harvest time (flowering period)	Essential oils
Acid index	2.86
Ester Index	22.44
Refractive index	1.43
Miscibility with ethanol (v/v)	1/1.5
Density at 20 °C	0.84

Quantitative analysis of essential oils using CG/SM

The chromatographic profile and the chemical composition of EOs are shown respectively in Fig. 2 and Table-2 where all various components are ordered according to their elution times from the chromatographic column.

From Fig. 2 and Table-2, it has been clearly shown that the aerial part of *Ruta chalepensis* contains three major constituents such as 2-Undecanone (35.51 %), Decanol-2 methyl (8.62 %) and 2-Dodecanone (6.87 %). Similar results are stated by several previous studies [21, 25]. On contrary, it has been reported that the essential oils composition varied from a country to another. An

investigation on the same species harvested from Tunisia signaled 2-Undecanone (34.4-49.18 %), 2 Heptanol acetate (13.5-15.4 %) and α Pinene (9.8-11.9 %) as major compound [26]. The Turkish oils from *Ruta chalepensis* are characterized by the abundance of 2-Undecanone (43.2 %),2-Nonanone (27.9 %) and 2- Nonyl acetate (13.8 %) [27]. Moreover, in Cuba, 2-Undecanone (34.88 %), 2-Nonanone (25.23 %) and 1-Nonene (10.93 %) are considered as predominant compounds of the essential oils obtained from *Ruta chalepensis* [28] whereas in Ethiopia, 2-Undecanone (31.74 %), Dodecene (13.59 %) and 2-Nonanone (9.57 %) are signaled as major constituents of oils obtained from the same plant [23] in addition, it has been demonstrated that the oils of the same species collected from Palesteine are predominant by the following compounds ; Linalyl acetate (34.21 %) and 2-Undecanone (26.79 %) [29]. This difference in composition is influenced by both intrinsic and environmental factors such as geographic location, climatic conditions, soil conditions, trauma and diseases [21].

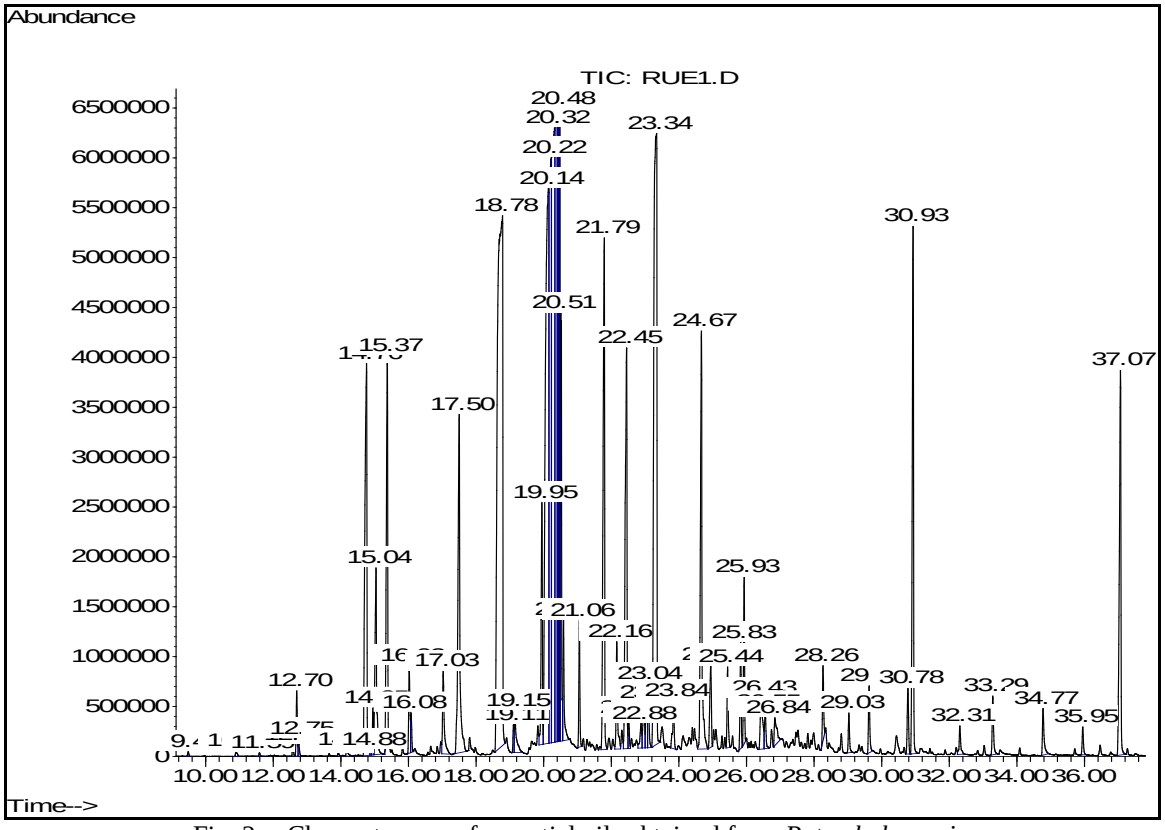

Fig. 2: Chromatogram of essential oils obtained from *Ruta chalepensis*.

Table-2: Chemical composition of essential oils obtained from *ruta chalepensis*

Compounds Number	Retention time (RT) (mn)	Retention Indices (RI)	Composition	Amount Percentage
01	9.476	915	α-pinene	0.024
02	10.899	941	β-Phellandrene	0.04
03	11.591	955	β-Myrcene	0.01
04	12.579	972	Benzene, 1-metyyl-4- (1-methylethyl)-	0.05
05	12.700	975	D-Limonene	0.32
06	12.751	976	Eucalyptol	0.09
07	14.163	1003	1-Octanol	0.06
08	14.711	1022	2-Nonanone	3.66
09	14.883	1029	Terpineol, Z-B	0.032
10	15.043	1034	2-Nonanol	1.47
11	15.357	1046	Thuyone	2.44
12	17.495	1123	2-Decanone	3.01
13	20.47	1241	2-Undecanone	35.51
14	20.507	1243	2-Undecanol	2.48
15	22.427	1325	2-Dodecanone	6.86
16	23.336	1365	1-Decanol-2 methyl	8.62
17	24.645	1425	Tridecanone	3.06

Biological activities

Antimicrobial activity evaluation

According to Table-3, it has been shown that the EOs of *Ruta chalepensis* react differently on all tested strains. Both strains of yeasts such as *S. cerevisiae* and *C. albicans* appear more sensitive to extracted oils than the others, with an inhibition diameters varied from 27 to 28 mm. These results are better to those obtained by [19] who has tested the oils of *Ruta chalepensis* against *Candida albicans* and has found consequently an inhibition zones varying from 12 to 18 mm whereas other authors such as [24, 30] have reported respectively an inhibition zones of 11 mm and 15 mm using the oils of the same species. These findings are in concordance with those reported in numerous previous studies which have revealed an effective antifungal activity of EOs relative to the tested plant [20, 21].

Table-3: The minimum inhibitory concentration of the tested microorganisms.

Tested strains	ZI (mm)	MIC(%)	MBC or MFC (%)
Bacteria (Gram $^-$)			
***Escherichia coli* ATCC4157**	**22**	**>2**	**-**
***Klebsiella pneumoniae* ATCC4352**	**25**	**0.125**	**0.5**
Bacteria (Gram $^+$)			
***Bacillus subtillis* ATCC9372**	**24**	**0.125**	**0.125**
***Staphylococcus aureus* ATCC6538**	**24**	**1**	**1**
Fungi			
***Candida albicans* ATCC24433**	**28**	**< 0.03**	**0.125**
***Saccharomyces cerevisiae* ATCC2601**	**27**	**< 0.03**	**0.06**

ZI: Zone Inhibition

Concerning the tested bacterial strains, *K. pneumoniae* is found to be sensitive to the essential oils with an inhibition zone of 25 mm compared to *B. subtillis*, *S. aureus* and *E. coli* which have all an inhibition diameter in the interval of 22 to 24 mm. These results are correlated with those obtained with [31] who have showed that the EOs of *Ruta chalepensis* have a moderate effect on *S. aureus* ATCC25923 and *P. aerogenosa* ATCC27853 whereas they are better to those reported by [32] who have signaled an inhibition effect of *Ruta chalepensis*'oils towards *K. pneumonia* ATCC27853 (20 mm), *K. pneumonia* HS (18 mm),*S. aureus* ATCC 2913 (17 mm), *S. aureus* HS (14 mm), *E coli* ATCC 25922 (20 mm) and *E.coli* HS (20 mm).On contrary, these results are in disagreement with those obtained with some authors [33] who have signaled that the EOs from *Ruta chelepensis* have a negative reaction against *E. coli*. In addition ,in another previous study [30], it has been demonstrated a lower antibacterial activity of Tunisian oils from *Ruta chalepensis* towards *E.coli* (7 mm), *K. pneumonia* (6 mm) and *S. aureus* (17 mm).On the basis of several previous investigations, the antibacterial activity is better against Gram positive bacteria than Gram negative one [34]. This difference could be attributed to the presence of hydrophilic outer membrane surrounding the cell wall membrane which blocked further the penetration of hydrophobic essential oils [35]. On contrary, it has been stated that *E. coli* (Gram -) has a stronger inhibition against EOs of one variety of *Ruta chalepensis* comparing to bacteria (Gram +) [19].

In the case of MIC, the lowest MIC value is observed with *C. albicans* and *S. cerevisiae* at a value less to 0.03 % (v/v) followed by *S. aureus* with a concentration more than 1 % (v/v) and further *B. subtillis* and *K. pneumoniae* at value of 0.125 % (v/v). However *E. coli* is considered less sensitive than the other microorganisms because it has presented a highest MIC value more than 2 % (v/v).From the Table-3, it has been illustrated also that *S. cerevisiae* has the lowest MFC value at 0.06 % (v/v). This strain is followed by *C. albicans* (MFC value of 0.125 %), *B. subtillis* (MBC value of 0.125 %) and *K. pneumoniae* (MBC value of 0.5 %). The strongest MBC value is obtained with *S. aureus* at concentration of 1 % (v/v).

Subsequently, the essential oils of *Ruta chalepensis* exert a good antimicrobial activity against the six strains used in this study, where we notice a little variation between the obtained result and those of several authors indicating a best inhibitory activity with oils of *Ruta chalepensis* [14, 19, 33].

Moreover, the effect of EOs on microorganisms differs from plant to another. It depends on their type, composition and concentration [36].These oils efficacy could be probably linked to the presence of ketones substances such as 2-Undecanone, 1-Decanol 2-methyl and 2-Dodecanone. Some authors [37] have signaled the important role of ketones containing in EOs obtained from *Mentha puleguim* in eventually good antimicrobial activity. On contrary, other authors [38] have cited that the majors components of EOs thought to play a more significant role in the antimicrobial activity while the minor constituents are thought to result in synergistic outcomes.

Table-4: Parameters of anti-inflammatory activity.

	Average weight (g).		% of edema increase	% of edema inhibition relative to control
	Left leg	Right leg		
Control	**0.204 ± 0.025**	**0.154 ± 0.0078**	**32**	**-**
Essential oils	**0.1291 ± 0.0137**	**0.1124 ± 0.0149**	**14.85 ***	**53.59**
Diclofenac	**0.160 ± 0.01**	**0.138 ± 0.0139**	**15.94***	**50.18**

Anti-inflammatory activity

From Table-4, it has been observed that the percentages of edema increase in the left legs comparing to the right one. In control, sample and reference groups are respectively 32 %, 15.85 % and 14.85 %. Indeed, the carrageen induce edema has been commonly used as an experimental animal model for acute inflammation which is believed to be biphasic. The first phase (1-2 h) is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surrounding. The second one is sustained by prostaglandin release and mediated by bradykinin, leukotriene and polymorphonuclear cells [39]. As indicated in Table-4, the *Ruta chalepensis*' oils at dose of 0.5 ml/mice have a significant reduction of inflammation induced by carrageen injection. Subsequently, the percentage of edema reduction in mice treated with EOs versus controls is 53.59 % ($p < 0.004$) while the edema suppression percentage with Diclorofenac versus controls is 50.46 % ($p < 0.019$). This decrease would be related to the inhibition of inflammation mediator such as serotonin, prostaglandin and histamine. This activity has not been studied before with EOs obtained from *Ruta chalepensis* but it is tested on oils from others species belonging to the same genus. Our findings are more significant to those obtained from oils of *Ruta graveolens* (Mannenmacher et *al*, 2017) [40].

To give more explication to this phenomena, a previous study has proved that EOs have an effect on lipopolysaccharides stimulated RAW 264.7 macrophage cells. They inhibit nitric oxide and prostaglandin E_2 production and suppress the lipopolysaccharides induced expression of cyclooxygenase-2 protein. Furthermore, EOs regulate the production of inflammatory cytokines, tumor necrosis factor, α interleukins IL6 and IL-1B [41].On general, the anti-inflammatory properties are related to chemical composition of EOs .They could be due to ketene substances such as 2 Undecanone, 1Decanol-2 methyl and Dodecanone. Similarly,a comparative study using sodium Houttuyfonate as agent of anti-inflammatory and pure 2 undecanone has also demonstrated in vivo their potent anti-inflammatory properties [42].

Conclusion

The extraction efficiency of the essential oils obtained from the aerial part of *Ruta chalepensis* has given a yield of 0.40 ± 0.03 % (w/w) during 4 hours. According to the physicochemical analyzes, these EO_S are characterized by an adequate quality compared to the literature with the following parameters: acid index; 2.86, ester index; 22.44, refractive index; 1.43 and density of 0.84. Furthermore, both qualitative and quantitative analyses of oils using GC-MS have identified seventeen volatile compounds with the abundance of ketones compounds representing by 2-undecanone (35.51 %), 1- Decanol-2 methyl (8.62 %) and 2-Dodecanone (6.86 %).The study of the antimicrobial activity using the diffusion method on solid medium revealed that these oils have a strong inhibitory action against all the tested strains particularly the yeasts *Candida albicans* and *Saccharomyces cerevisiae* , with the same MIC value inferior to 0.03 % (v/v).On the other hand, the *Ruta chalepensis*' oils have proved in vitro a good anti-inflammatory activity with edema reduction rate of 53.59 %.

References

1. J. B .Calixto, M. F. Otuki and A. R. Santos, Anti-inflammatory compounds of plant origin. Part I. Action on arachidonic acid pathway, nitric oxide and nuclear factor *κ* B (NF-*κ*B). *Planta .Med*., **69**, 973 (2003).
2. K. D. Rainsford, Anti-inflammatory drugs in the 21st century. *Subcell.Bioch*., **42**, 3 (2007).
3. A. Pilotto, M. Franceschi, G. Leandro, F. Paris,V. Niro, M.G. Longo, L. P. D'Ambrosio, A. Andriulli and F. Di Mario, The risk of upper gastrointestinal bleeding in elderly users of aspirin and other non-steroidal anti-inflammatory drugs: the role of gastroprotective drugs. *Aging. Clin .Experim .Res.*, **15**,494 (2003).
4. J. B. Calixto, M. M. Campos, M. F. Otuki and A. R. S. Santos, Anti-inflammatory compounds of plant origin. Part II. Modulation of pro-inflammatory cytokines, chemokines and adhesion molecules. *Planta.Med.*, **70**, 93 (2004).
5. M. B. Kasimala, M. Tukue and R. Ermias, Phytochemical screening and antibacterial activity of two common terresterial medicinal plants *ruta chalepensis* and *rumex nervosus*. *Bali Med. J*, **3**, 116 (2014).
6. A. Pollio, A. De Natale, E. Appetiti, G. Aliotta and A. Touwaide, Continuity and change in the Mediterranean medical tradition: *Ruta* spp. (Rutaceae) in Hippocratic medicine and present practices. *J. Ethnopharmacol*., **116**, 469 (2008).
7. A. Khadraoui, A. Khelifa, H. Boutoumi, H. Hamitouche, R. Mehdaoui1, B. Hammouti, S. S. Al-Deyab, Adsorption and Inhibitive Properties of Ruta chalepensis L. Oil as a Green Inhibitor of Steel in 1 M Hydrochloric Acid Medium, *Int. J. Electrochem. Sci.*, **9**, 3334 (2014).

8. E. Bejar, R. Bussmann, C. Roa and D. Sharon, Herbs of Southern Ecuador. A Field Guide to the Medicinal Plants of Vilcabamba. *Rodale Press*, 256 (1997).
9. E. San Miguel, Rue (ruta L., rutaceae) in traditional Spain: frequency and distribution of its medicinal and symbolic applications., *Econ. Bot*, **57,** 231 (2003).
10. M. Kacem, I. Kacem, G. Simon, A. Ben Mansour, S. Chaabouni, A. Elfeki and A. Bouaziz, Phytochemicals and biological activities of *Ruta chalepensis L*. growing in Tunisia. *Food Biosci* (2015).
11. V. Hammiche and M. Azzouz, Les rues: Ethnobotanique, phytopharmacologie et toxicité, *Phytothérapie*, **11**, 22 (2014).
12. A. A. Al Qarawi, Stimulatory effect of the aqueous extract of *Ruta chalepensis* on the sex organs and hormones of male rats. *J. Appl. Res.*, **5,** 206 (2005).
13. N. Bozin, M. Dukic, Simin and G. Anackov, Characterization of the volatile composition of essential oils of some Lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils, *J. Agric.Food.Chem.*,**54**, 1822 (2006).
14. T. Amdouni, S. BenAbdallah, N. Msilini, F. Merck, M. Chebbi, M. Lachâal, N. Karray-Bouraoui, Z. Ouerghi and X. Fernandez, Effect of salt stress on the antimicrobial activity of *Ruta chalepensis* Essential oils, *Acta.Physiol.Plantarum.*, **38**, 147 (2016).
15. AFNOR (Association Française des Normalisations, Recueil de norme française sur les huiles essentiells). Tome I. 2ème Ed, paris, 45 (2000).
16. H. Van den Dool and P. D. Kratz, A generalization of the retention index system including linear temperature programmed gas liquid partition chromatography.J. *Chromatogr*., **11**, 463 (1963).
17. A. G. Ponce, R. Fritz, C. Del Valle and S. L Roura, Antimicrobial activity of essentiel oils on the native microflora of organic Swiss chard. *Libensmittel-wissenchaftund Technol.*, **500** (2003).
18. C. A. Winter, E. A. Risley and G. W. Nuss, Carrageenan-induced oedema in the hind paw of rat as an assay for anti-inflammatory activity, *Proc.Soc. Exp. Biol. Ther*. **111**, 544 (1962).
19. A. Muhammad, I. Anis, A. Khan, B. P. Marasini, M. I. Choudhary, M. R. Shah, Biologically Active *C*-Alkylated Flavonoids from *Dodonaea viscose*, *Arch. Pharmacal. Res.***, 35**, 431 (2012).
20. M. Khoury, M. El Beyrouthy, N. Ouaini, M. Iriti, V. Eparvier and D. Stien, Chemical composition and antimicrobial activity of the essential oil of *Juniperus excelsa M. Bieb*. growing wild in Lebanon, *Chem. Biodivers.*,**11**, 825 (2014).
21. S. Merghache, M. Hamza and B. Tabti, Etude physicochimique de l'huile essentielle de *Ruta chalepensis* L. De Tlemcen, Algérie, *Afrique Sci.*, **05**, 67 (2009).
22. S. Fellah, M. Romdhane et M. Abderraba,Valorisation de l'huile essentielle de la *Salvia officinalis* de Tunis, cueillie dans deux régions différentes de la Tunisie, *J. Soci. Alg .Chim.*,**16**, 193 (2001).
23. K Defa, A. J Sekarathil, G. Shiferaw, S. Feleke and E. Gurmesa, Extraction, Optimization and Characterization of the Ethiopian *Ruta Chalepensis* Crude Oil. Inter. *J.Chem .Biomed Sci*, **4,** 11 (2018).
24. M. Ayaz, M. Junaid, F. Ullah, A. Sadiq, M. A. Khan, W. Ahmad, M. R. Shah, M. Imran, S. Ahmad, Comparative chemical profiling, cholinesterase inhibitions and antiradicals properties of essential oils from Polygonum hydropiperL: A Preliminary anti- Alzheimer's study, *Lipids in Health and Disease*, **14**, 141 (2015).
25. M. Ferhat, A. Kabouche and Z Kabouche, Comparative compositions of essential oils of three Ruta species growing in different soils, *J. Mater. Environ. Sci*. **5**, 735 (2014).
26. W. Bouabidia, M. Hananab, S. Gargouric, I. Amrid, T. Fezzanie, M. Ksontinie, B. Jamoussif and L. Hamrouni, Chemical composition, phytotoxic and antifungal properties of *Ruta chalepensis L.* essential oils, *Nat. Product Res*, **1** (2014).
27. S. B. Khan, H. Azhar ul, S. Perveen, N. Afza, A. Malik, S. A. Nawaz, M. R. Shah, M. I. Choudhary, Butyrylcholinesterase inhibitory guaianolides from *Amberboa ramosa*. *Arch. Pharmacal. Res***, 28** , 172 (2005).
28. O. Pino, Y. Sánchez, M. M. Rojas, Y. Abreu, T. M. Correa, D. Martínez, R. Montes de Oca, Chemical composition and antibacterial activity of the essential oil from *Ruta chalepensis* L. *Revista de Protección Vegeta*l, **29**, 220 (2014).
29. N. Jaradat, L. Adwan, S. K'aibni, A. N. Zaid, M. J. Y. Shtaya, N. Shraim, and M. Assali, Variability of chemical compositions and antimicrobial and antioxidant activities of *Ruta chalepensis* Leaf Essential Oils from Three Palestinian Regions, *Hindawi BioMed Res Inter* (2017).
30. F. Haddouchi, T. M. Chaouche,Y. Zaouali, R. Ksouri, A. Attou and A. Benmansour, Chemical composition and antimicrobial activity of the essential oils from four Ruta species growing in Algeria, *Food.Chem.*, 141, 253 (2013).

31. L. El Bouzidi, A. Abbad, L. Hassani, K. Fattarsi, D. Leach, M. Markouk, L. Legendre and K. Bekkouche .Essential Oil Composition and Antimicrobial Activity of Wild and Cultivated Moroccan *Achillea ageratum* L. a Rare and Threatened Medicinal Species, *Chem.Biodiv*. **9**, 598 (2012).
32. S. Chibani, A. Bouratoua, A. Kabouche, S. Laggoune, Z. Semra, F. Smati and Z. Kabouche Z, Composition and antibacterial activity of the essential oil of *Ruta chalepensis* subsp. *angustifolia* from Algeria, *Der Pharmacia Lettre*,**5**, 252 (2013).
33. E. Ben Bnina, S. Hammami, M. Daamii-Remadi, H. Ben Jannet and Z. Mighri Z, Chemical composition and antimicrobial effects of Tunisian *Ruta chalepensis L*' essential oils, *J. Soc. Chim. Tunis.*, **12**, 1 (2010).
34. M. Gilles, J. Zhao, M. An and S. Agboola, Chemical composition and antimicrobial properties of essential oils of three Australian Eucalyptus species, *Food. Chem.,* **119**, 731 (2009).
35. S. Burt. Essential oils: their antibacterial properties and potential applications in foods-a review, *Int .J . Food. Microbiol*., **94**, 223 (2004).
36. M. Marino, C. Bersani, and G. Comi, Impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae, *Inter. J. Food Microbiol*., **67**, 187 (2001).
37. L. Belghazi, N. Lahlou, I. Alaoui, T. Abousaouiria, N. Habti, I. A. Tantaoui and K. Fellat-Zarrouck. Extraction et analyse par chromatographie en phase gazeuse de l'huile essentielle de la menthe pouliot. Test antifongique [Extraction and analysis by gas chromatography of essential oil of pennyroyal mint tested as antifungal]. *Congrès de biochimie. Casablanca. Biochimie et santé*., 38 (2002).
38. W. Li, Q. Shi, Q. Liang, X. Xie, X. Huang and Y. Chen, Antibacterial activity and kinetics of *Litsea cubeba* oil on *Escherichia coli*, *Plos. One*., **9**, 1 (2014).
39. M. Gupta,U. K. Mazumder, P. Gomathi and V. Thamilselvan, Antiinflammatory evaluation of leaves of *Plumeria acuminate*, *BMC Complem. Alter. med.*, **36**, 1472 (2006).
40. J. L. Nonnenmacher, B. S.Mikulski, S. S. Roman, Anti-inflammatory activity of the essential oil and hydroalcoholic extract of *Ruta graveolens* l. (Rue) on ear edema in mice. *Perspectiva, Erechim*, **41**, 125 (2017).
41. M. J. Kim, K. W. Yang, S. S. Kim, S. M. Park, K. J. Park, K. S. Kim, Y. H. Choi, K. K Cho, N. H. Lee, C. G. Hyun, Chemical composition and anti-inflammatory effects of essential oil from hallabong flower, *Excli. J*., **12**, 933 (2013).
42. J. Chen,W. Wang, C. Shi and J. Fang, A Comparative Study of Sodium Houttuyfonate and 2-Undecanone for Their *in Vitro* and *in Vivo* Anti-Inflammatory Activities and Stabilities, *Int .J. Mol. Sci*.,**15**, 22978 (2014).