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Original Research Article

Evaluation of the Antioxidant, Antifungal and Anticholinesterase activity of the Extracts of *Ruta Montana* L., Harvested from Souk-Ahras (North-East of Algeria) and Composition of its Extracts by GC-MS

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ABSTRACT

Ruta montana is a wild medicinal plant traditionally used by ancient populations to treat various diseases The aimed is to evaluate the antioxidant, antifungal and anti-cholinesterase activities of six extracts [Essential oil (01), Soxhlet extract (02), the hexane extract by maceration (03), the extract Chloroform (04), the extract with ethyl acetate (05) and the butanol extract (06)], obtained from the aerial part of Ruta montana L., harvested from Souk-Ahras North-East from Algeria. For the antioxidant activity, seven tests were used. The results, all tests included, confirm that the extracts of Soxhlet (02), chloroform (04), ethyl acetate (05) and butanol (06) have the best antioxidant activity with IC_{50} = 173.54±7.27 µg/ml, $A_{0.5}$= 46.21±3.99 µg/ml, $A_{0.5}$= 35.06±0.49 µg/ml and IC_{50}= 59.48±7.79 µg/ml respectively. For the evaluation of the antifungal activity, it was found that the chloroform extract (04) gave better antifungal activity against the fungus (Fusarium oxysporum f. sp. lycopersici (FOL)) with a fungistatic effect which is translated to an inhibition rate of 11.91% at 1mg and 60.91% at 40mg. The composition of essential oil (01), of Soxhlet extract (02) and hexane extract (03) was evaluated by GC-MS with a major composition of 2-nonanone (24.93%), 2- undecanone (22.62%) and Acetic acid, non-2-yl ester (14.15%), for the essential oil; hydrocarbons (56.89%), 2-undecanone (10.53%), 2-nonanone (9.24%) and the alkaloid Dictamnine,6,7-dimethoxy with a rate of (8.39%), for soxhlet extract (02) and for hexane extract (03) the hydrocarbons are present with a rate of (47.19%) followed by 2-nonanone (13.31%) and 2-undecanone with a rate of 8.44%.

***Keywords*:** Ruta montana, antioxidant activity, antifungal activity, anti-cholinesterase activity, essential oil, GC-MS .

Introduction

Medicinal plants play a significant role in healthcare, both in ancient and modern cultures.[1-3] The production of medicines derived from plants is based on the fact that they serve as important sources of therapeutic agents. They are preferred due to their availability, acceptable cost, and non-toxic nature compared to modern medicine.[4] *Ruta* is a medicinal plant genus that comprises over 1800 species.[5] These shrubby plants originated in the Mediterranean region, but their distribution has expanded to include hot areas and tropics.[6] The most common species are *Ruta chalpensis* L., *Ruta graveolens* L., and *Ruta montana* L. *Ruta* has been utilized in traditional medicine for the treatment of various diseases. It is employed as a stimulant, abortifacient, anti-inflammatory, resolving agent, for eye problems, dermatitis, rheumatic medicine, hypertension treatment, phototoxicity, bacteriostatic effects on skin diseases, and rhinitis.[7]

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Ruta species serve as sources of diverse classes of natural products with biological activities, including antifungal, antioxidant, phytotoxic, abortifacient, depressant, anti-inflammatory, and antidote properties.[8] Many researchers have shown interest in studying *Ruta* species worldwide for a considerable period. In 2000, Touati and his colleagues identified alkaloids from *Ruta montana* in Morocco,[9] while in 2003, Kabouche and his team discovered a new dicoumarinyl ether and two rare furocoumarins from *Ruta montana* in Mila (northeast Algeria).[10] Due to the abundant presence of alkaloids and antioxidants in *Ruta* species,[11] several researchers have focused on evaluating their antioxidant and antibacterial activities.[12-15] Phytochemists have also targeted the analysis of *Ruta*'s essential oil, which is rich in antioxidants.[16] Furthermore, other effects of *Ruta* extracts have been tested, including their antidiabetic effect,[17] antiacetylcholinesterase effect,[18] and insecticidal effect.[19] The majority of studies conducted on *Ruta* species have originated from North African countries such as Algeria, Morocco, and Tunisia.

The objective of the present study is to assess the antioxidant activity of six different extracts, including the essential oil, using seven different methods. Additionally, the antifungal activity and anticholinesterase activity (AchE) of a *Ruta montana* specimen harvested from eastern Algeria (Souk Ahras) will be evaluated. The composition of three extracts, namely the essential oil (01), soxhlet extract (02), and hexane extract (03), will be identified using gas chromatography coupled with Flame Ionization Detector and mass spectrometry. This study offers a useful contribution in terms of biological activities data of autochthonous north African plants. A multitude of tests have been performed and the results may be of interest to the African phytochemical research community

Material and Methods

Plant material

The aerial parts of *Ruta Montana*, including the stem and leaves, were harvested in the commune of Oued Elkebrit, wilaya of Souk Ahras (coordinates: 35°55'36.5" N, 7°54'40.4" E) at the end of January 2020. The plant material was identified by Professor Eddoud from the Department of Biology at the University Kasdi Merbah-Ouargla (UKMO) and was deposited in the herbarium of the National Polytechnic School of Constantine with the voucher number RM0011/01/2020. Subsequently, the freshly harvested plant was dried in the shade in a dry and well-ventilated location for a period of 10 days. It was then cut into thin pieces for further processing.

Preparation of extracts

Extraction of the essential oil

The essential oil (01) of the plant under study was extracted using a modified version of the process described by Amarti *et al.*[20] Hydrodistillation was performed using a Clevenger-type apparatus, where 350 g of fresh plant material was mixed with 2/3 L of distilled water in a 2L flask. The mixture was heated for 2 hours and 30 minutes. After the extraction, the essential oil was separated from the water and stored in a dark environment with the presence of a desiccant (anhydrous sodium sulfate, Na_2SO_4) to ensure its preservation.

Soxhlet extraction

Soxhlet extraction (2), also known as hot extraction, is a technique employed to achieve a relatively thorough extraction of metabolites. In this study, ethyl acetate (AcOEt) was utilized as the solvent for extraction.

The extractor used in the process consists of a cartridge filled with 90g of plant material. This cartridge is attached to a flask that contains 600ml of the solvent. The extraction process involves multiple cycles, typically seven, during which the solvent continuously circulates through the cartridge to extract the desired compounds.

After completing the extraction cycles, the solvent is collected, typically with a dark green coloration, and then subjected to further processing. The next step involves passing the solvent through a rotary evaporator, under reduced pressure at a temperature of 45°C. This process facilitates the evaporation of the solvent, leaving behind a dry residue containing the extracted compounds.

Delipidation (03)

The purpose of this step is to eliminate lipids and pigments from the plant material. It involves macerating the plant material in hexane for duration of 24 hours. Hexane, being a solvent rich in lipids, aids in the removal of these lipid-based components from the plant material. After the maceration process, the solvent, now enriched with the extracted lipids, is collected. Subsequently, the solvent is evaporated to remove the hexane, leaving behind the lipids as a residue. This step helps in obtaining a purified extract of the desired compounds by eliminating unwanted lipids and pigments.

Maceration (cold extraction)

Maceration is a process that involves prolonged contact at room temperature, which helps to preserve heat-sensitive substances, between the plant material and a solvent for extracting the active ingredients. In this study, the process begins by depositing 340g of the pulverized plant material in 3L of 70% methanol for a duration of 24 hours. After the maceration period, the maceration solution is collected for further fractionation using liquid-liquid extraction. This extraction process is carried out using a separatory funnel and a series of organic solvents are used in order of increasing polarity: chloroform (04), ethyl acetate (05), and n-butanol (06). Each solvent is used until the aqueous phase becomes exhausted, indicating that no more extraction is taking place. The recovered organic phases from each solvent are then subjected to vacuum evaporation at a temperature of 45°C. This process helps to remove the solvents and achieve a dry state for each extract, resulting in concentrated fractions of the desired compounds.

Evaluation of antioxidant activity

DPPH test

The anti-radical activity was assessed using the DPPH assay, following the method outlined by Blois.[21] The assay was conducted in a 96-well microplate, with each well containing a volume of 200 µL. Initially, 40 µL of the extract was added to 160 µL of DPPH solution. The mixture was then incubated in the dark for a specific period of time. Subsequently, the absorbance was measured at 517 nm using a spectrophotometer.

For the preparation of the DPPH solution, 4 mg of DPPH was dissolved in 100 ml of methanol to obtain a solution with an absorbance of 0.5 at 517 nm. This solution served as the reagent for the DPPH assay.

ABTS Test

The cationic ABTS solution is prepared by combining two solutions: solution (01) of ABTS at a concentration of 7 mM and solution (02) of potassium persulfate at a concentration of 2.45 mM. These two solutions are mixed together and allowed to react for a period of 12 to 16 hours at room temperature, while being kept away from light.[22] Before using the mixture, it needs to be diluted until an absorbance of 0.7 ± 0.02 at a wavelength of 734 nm is achieved. This diluted ABTS solution is then used in the subsequent steps. In a microplate, 160 µL of the diluted ABTS solution is added, followed by the addition of 40 µL of the extract. The mixture is allowed to stand for 10 minutes, after which the absorbance is measured at 734 nm using a spectrophotometer.

Galvinoxyl Radical Removal (GOR) Test

To determine the galvinoxyl radical scavenging activity of each extract at various concentrations, the method described by Shi et al.[23] is followed: a 96-well microplate is used. In each well, 160 µL of galvinoxyl solution at a concentration of 0.1 mM is added. Subsequently, 40 µL of the extract is added to each well. The total volume is then adjusted to 2 mL in each well. The microplate is incubated for 120 minutes to allow the reactions to occur. Following the incubation period, the absorbance at 428 nm is measured using a spectrophotometer. This procedure allows for the evaluation of the galvinoxyl radical scavenging activity of the extracts at different concentrations.

Reducing power (RP) test

The reducing power (RP) of the extracts is measured using a modified method based on the procedure described by Oyaizu *et al.*[24] Add 10 µL of the extract to a test tube. Then, introduce 40 µL of phosphate buffer solution with a pH of 6.6 into the same test tube. Next, add 50 µL of 1% potassium ferricyanide ($K_3Fe(CN)_6$) to the mixture within the test tube. Incubate the mixture at a temperature of 50°C for duration of 20 minutes. After the incubation period, add 50 µL of 1% trichloroacetic acid to the mixture, followed by 40 µL of distilled water. Add 10 µL of 1% $FeCl_3$ to the mixture and ensure thorough mixing of the contents in the test tube. Finally, measure the absorbance of the mixture at a wavelength of 700 nm using a spectrophotometer. The measurement of the absorbance at 700 nm provides an indication of the reducing power of the extracts.

Phenanthroline activity test

The phenanthroline activity is determined using the method described by Szydlowska-Czernaik.[25] Add 10 µL of the extract to a test tube. Then, add 50 µL of a 0.2% $FeCl_3$ solution (0.02 g of $FeCl_3$ dissolved in 10 ml of water) to the test tube. Next, introduce 30 µL of a 0.5% phenanthroline solution (0.05 g of phenanthroline dissolved in 10 ml of methanol) to the mixture in the test tube. Add 110 µL of methanol to the test tube and mix the contents thoroughly. Incubate the mixture in the dark for 20 minutes at a temperature of 30°C. After the incubation period, measure the absorbance of the mixture at 510 nm using a spectrophotometer. The absorbance measured at 510 nm provides information about the phenanthroline activity of the extracts.

Silver nanoparticle (SNP) test

The metal chelate activity is determined using the method described by Mustapha Özyorek.[26] To prepare the spherical silver nanoparticles (SNP) solution (S1), mix 50 ml of silver nitrate (1 mM) and 5 ml of

trisodium citrate (1%) in a suitable container. Slowly add the trisodium citrate solution drop by drop to the silver nitrate solution until a pale yellow colour is obtained, indicating the formation of spherical silver nanoparticles. In a test tube, add 20 µL of the extract, followed by 130 µL of the SNP solution (S1). Add 50 µL of water to the mixture in the test tube and thoroughly mix the contents. Incubate the mixture at 25°C for 30 minutes. After the incubation period, measure the absorbance of the mixture at a suitable wavelength using a spectrophotometer. The reading of the absorbance after the incubation period provides information about the metal chelate activity of the extracts

CUPRAC Test
The reduction capacity of cupric ions in the extracts at different concentrations is determined using the method described by Apak *et al*.[27] In a 96-well microplate, combine 40 µL of the extract with 40 µL of an acetate buffer solution containing ammonium at pH 7. Then, add 50 µL of an ethanolic solution of neocuprine to the mixture in the microplate. Next, introduce 50 µL of a $CuCl_2$ (Cu II) solution to the microplate. Thoroughly mix the contents of the microplate. Incubate the microplate for 1 hour to allow the reactions to occur. After the incubation period, measure the absorbance of the mixture at 450 nm using a spectrophotometer. The absorbance measured at 450 nm provides information about the reduction capacity of cupric ions in the extracts at different concentrations.

Anti-cholinesterase activity (AchE)
The AchE (acetylcholinesterase) activity is determined using the method described by Ellman *et al*.[28] Prepare a microplate with 96 wells, ensuring that each well contains 150 µL of sodium buffer solution at pH 8. Add 10 µL of the extract, which should be dissolved in ethanol at different concentrations, to each well of the microplate. Then, add 20 µL of the AchE solution to each well. Incubate the microplate for 15 minutes at 37°C to facilitate the reactions. After the incubation period, add 10 µL of dithio-bis(2-nitrobenzoic) acid (DTNB) at a concentration of 0.5 mM and iodide acetylthocholine at a concentration of 0.71 mM to each well of the microplate. Take an initial reading at t=0 minutes at 412 nm using a spectrophotometer. Re-incubate the microplate for an additional 15 minutes. Following the second incubation, take a final reading at t=15 minutes at 412 nm using the spectrophotometer. By comparing the absorbance values at t=0 minutes and t=15 minutes, the AchE activity of the extracts can be determined.

Antifungal activity
To test the inhibitory effect of the extracts, the fungus *Fusarium oxysporum f. sp. lycopersici* (FOL), which causes vascular wilt of tomatoes, was used.[29] The protocol followed for determining the fungicidal effect is based on the method described by Song *et al*. and involves using PDA (Potato Dextrose Agar) as the culture medium.[30] Prepare a solution of DMSO (Dimethyl sulfoxide) with various concentrations of the extracts (1 mg/ml, 2 mg/ml, 4 mg/ml, 8 mg/ml, 16 mg/ml, 40 mg/ml). Add 1 ml of the DMSO solution to 100 ml of PDA (Potato Dextrose Agar) at 60°C and mix thoroughly. Under aseptic conditions, distribute the prepared mixture into four sterile petri dishes. Create a positive control by adding 1 ml of DMSO alone to the PDA, and a negative control by using PDA alone. Place a 5 mm diameter disc of *Fusarium oxysporum f. sp. lycopersici* (FOL) in the center of each petri dish. Incubate the petri dishes at 25°C for a period of six days. After incubation, the inhibitory effect of the extracts can be evaluated by examining the growth of the fungus around the disc and comparing it to the positive and negative controls. The inhibition rate is calculated by the formula of Denis *et al*.[31]

$$I\% = \frac{C-T}{C} \times 100$$

T: growth diameter of the different concentration.
C: positive control growth diameter.

GC-FID and GC-MS analysis
3 of our extracts: essential oil (01), Soxhlet extract (02) and hexane extract (3) underwent chromatographic analysis by GC-FID and GC-MS to identify their composition.

GC-FID: Essential oil components were analysed using a SCHIMADZU GC-2010 chromatograph equipped with Rxi-5ms capillary column (30 m * 0.25 mm, film thickness 0.25 µm). Helium was the carrier gas, at a flow rate of 1.44 ml/min. The oven temperature was maintained at 45°C for 10 min and then increased to 180°C at a rate of 3°C/min and maintained at 180°C for 5 min, then to 280°C at a rate of 5°C/min and maintained at 280°C for 5 min and finally to 330°C at a rate of 10°C/min for 2 min. Injector and detector (FID) temperatures were set at 330°C. Diluted samples (in dichloromethane) of 1 µl were injected in the split/splitless (30:1 split) mode.
GC-MS analysis was performed using a GCMS-QP2010. The GC Conditions are the same used in GC-FID analysis. In GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Injector temperature was: 330°C. Diluted sample (in dichloromethane) of 1 µl were injected in the split/splitless (30:1 split) mode.
The identification of oil components obtained from the capillary column was performed by comparing their retention index (KI), calculated from GC-FID analysis, with those reported in literature.[32] Additionally, their mass fragmentation patterns were compared with those available in databases for further confirmation.

Statistical analysis
The results of the tests carried out are expressed as the mean ± SD of analysis in three tests. The values of IC_{50} (50% inhibition concentration) and of $A_{0.5}$ (the concentration indicating 0.50 of absorbance) are calculated by the linear regression method from the two curves: [% inhibition = f (concentrations)] for IC_{50} and [Absorbance= f (concentrations)] for $A_{0.5}$.

Results and Discussion

Yield of essential oil
The essential oil of the aerial part of *Ruta Montana* studied has a yield of 0.67%, the latter is between the yields found by Mohammedi with 0.38% collected from Tiziouzou and 0.45% of Djelfa but the yield is lower than that found by Zellagui with a yield of 4.5% of the plant harvested from Oum el Bouaghi and 1% from Mila.[33-34] It is worth noting that variations in essential oil yield can occur due to several factors, including the plant's geographical location, climatic conditions, harvesting time, and extraction method used. These differences in yield could be attributed to the specific characteristics of the plant material and the extraction conditions employed in each study.

Evaluation of antioxidant activity
To carry out this evaluation, 4mg of each plant extract were dissolved in 1ml of ethanol.

DPPH test
The DPPH test results are presented in Table 1, revealing that the essential oil's IC_{50} value exceeds 800 µg/ml. Meanwhile, the extracts demonstrate IC_{50} values of 169.18±3.21 µg/ml, 84.01±6.40 µg/ml, 526.07±6.52 µg/ml, and 91.07±0.67 µg/ml for extracts 04, 02, 06, and 05, respectively. It is observed that extract 03, which is the delipidation extract, has no inhibition effect; our results are superior to the standards used (BHA, BHT, α-Tocopherol) and even superior to the results obtained by Kathirvel *et al*.,[35] who worked on *R. graveolens* (southern India) with an IC_{50}=19.50 ±0.03µg/ml. Ouerghemi *et al*.[36] and Bensghaier *et al*.,[5] who worked on *R. chalepensis* have found IC_{50}=30.69±0.041µg/ml and IC_{50}=54.50±1, 5µg/ml respectively.

ABTS Test
The results presented in Table 2 indicate that the calculated IC_{50} values exceed those of the standards used (BHA, BHT). Additionally, the essential oil (01) and delipidation (03) extracts exhibit IC_{50} values higher than 800 µg/ml. However, the IC_{50} values of extracts 02, 06, 04, and 05 are listed in descending order as follows: 173.54±7.27 µg/ml, 59.48±7.79 µg/ml, 46.54±3.45 µg/ml, and 36.22±2.59 µg/ml, respectively. The last four results are comparable with the IC_{50}=48.32±0.01µg/ml found by Kathirvel *et al*.[35]

Galvinoxyl Radical Removal (GOR) Test

According to the values of the IC_{50} (µg/ml) grouped in Table 3, we note that the essential oil (01) is not active and that the extract (06) gave better activity with the IC_{50}=125.02 ±1.67 µg/ml followed by the activities of the results of the extracts (05)= 146.06±6.08 µg/ml; (04)= 380.15±0.33 µg/ml, (03)= 378.40±0.75 µg/ml and (02) with IC_{50}=394.52±1.76 µg/ml .

The results of the three previous tables confirm that the extracts of Soxhlet, chloroform, ethyl acetate and butanol have better antioxidant activity.

Reducing power (RP) test

For the measurement of the reducing power of the extracts (Table 4), it was found that the essential oil has no activity and that only chloroform is active with $A_{0.5}$=134.40±4.67µg/ml. The rest of the extracts have $A_{0.5}$ higher than 200µg/ml.

Phenanthroline activity test

The appearance of the orange-red colour indicates the formation of the stable complex Fe^{2+}reduced with phenanthroline. The results obtained from this test show that the least active Soxhlet and haxane with $A_{0.5}$ = 181.44±7.66µg/ml and 200µg/ml respectively with an inactive essential oil but the extracts 04 and 06 have a moderate activity (46.21±3.99µg/ml and 60.40±0.69 µg/ml respectively); extract 05 has better activity with $A_{0.5}$ =35.06±0.49µg/ml. (Table 5)

Silver nanoparticle (SNP) test

According to results in Table 6, it can be seen that the essential oil has no activity and that the Sohxlet extract and hexane have an activity greater than 400 µg/ml; the activities of chloroform extract, butanol and ethyl acetate are respectively 296.09±1.02 µg/ml; 356.67±0.52 µg/ml and 456.96±2.78 µg/ml; these extracts have low activity compared to the activity of the standards used.

Table 1: % Inhibition in DPPH assay

Extracts	% Inhibition in DPPH assay							
	12.5 µg	**25 µg**	**50 µg**	**100 µg**	**200 µg**	**400 µg**	**800 µg**	**IC_{50} µg/mL**
(01)	NA	NA	NA	NA	NA	8.93 ± 0.48	13.28 ± 2.77	>800
(02)	3.52 ± 0.65	7.28 ± 3.76	8.18 ± 1.30	18.90 ± 1.11	23.81 ± 1.56	43.78 ± 1.95	59.74 ± 1.92	526.07 ± 6.52
(03)	NA	NA	NA	NA	NA	NA	NA	NA
(04)	NA	NA	19.08 ± 3.50	38.55 ± 3.55	55.65 ± 0.26	65.04 ± 0.58	Sat	169.18 ± 3.21
(05)	16.11 ± 2.73	22.90 ± 1.18	37.79 ± 2,67	60.38 ± 0,61	73.36 ± 0.48	Sat	Sat	84.01 ± 6.40
(06)	9.17 ± 1.13	17.92 ± 0.75	32.65 ± 1.21	61.62 ± 0.93	77.00 ± 0.28	78.64 ± 0.49	Sat	91.07 ± 0.67
BHA	76.55 ± 0.48	79.89 ± 0,26	81.73 ± 0,10	84.18 ± 0,10	87.13 ± 0,17	89.36 ± 0,19	90.14 ± 0,00	6.14 ± 0.41
BHT	49.09 ± 0.76	72.63 ± 2.06	88.73 ± 0.89	94.00 ± 0.31	94.97 ± 0.08	95.38 ± 0.41	95.02 ± 0.23	12.99 ± 0.41
α-Tocopherol	37.21 ± 1.82	81.53 ± 1.51	89.23 ± 0.12	89.38 ± 0.19	89.45 ± 0.22	89.99 ± 0.23	89.52 ± 0.33	13.02 ± 5.17

Table 2: % Inhibition in ABTS assay

Extracts	% Inhibition in ABTS assay							
	12.5 µg	**25 µg**	**50 µg**	**100 µg**	**200 µg**	**400 µg**	**800 µg**	**IC_{50} µg/mL**
(01)	NA	NA	NA	NA	11.50 ± 2.02	19.51 ± 3.47	_	>800
(02)	10.68 ± 1.16	14.11 ± 2.08	22.94 ± 3.21	37.77 ± 6.67	52.15 ± 3.45	68.01 ± 8.97	86.81 ± 2.18	173.54 ± 7.27
(03)	3.71 ± 1.98	5.07 ± 0.68	3.81 ± 4.50	6.16 ± 3.00	6.92 ± 2.13	10.19 ± 0.62	37.55 ± 1.70	>800
(04)	19.62 ± 2.81	34.88 ± 3.35	57.33 ± 0.16	76.62 ± 2.14	83.32 ± 0.43	88.88 ± 0.99	Sat	46.54 ± 3.45
(05)	23.13 ± 7.97	41.41 ± 5.57	59.57 ± 2.64	78.53 ± 2.20	89.02 ± 1.01	Sat	Sat	36.22 ± 2.59
(06)	22.27 ± 4.48	34.91 ± 5.47	46.87 ± 4.88	70.12 ± 5.84	88.16 ± 1.38	91.72 ± 0.18	92.15 ± 0.11	59.48 ± 7.79
BHT	69.21 ± 0.40	78.23 ± 1.34	88.12 ± 1.28	88.76 ± 3.07	90.85 ± 1.74	90.95 ± 0.51	96.68 ± 0.39	1.29 ± 0.30
BHA	92.83 ± 1.42	94.68 ± 0.42	94.95 ± 0.90	95.32 ± 0.25	95.59 ± 0.47	95.83 ± 0.15	95.86 ± 0.10	1.81 ± 0.10

Table 3: % Inhibition in Galvinoxyl radical (GOR) scavenging assay

Extracts	% Inhibition in Galvinoxyl radical (GOR) scavenging assay							
	12.5µg	**25 µg**	**50 µg**	**100 µg**	**200 µg**	**400 µg**	**800 µg**	**IC_{50}µg/mL**
(01)	NA	NA	NA	NA	NA	NA	NA	NA
(02)	NA	NA	3.36 ± 0.36	6.01 ± 0.74	24.89 ± 2.88	50.65 ± 0.24	66.80 ± 1.99	394.52 ± 1.76
(03)	NA	NA	NA	0.16 ± 3.36	2.09 ± 1.17	55.82 ± 0.98	87.84 ± 0.75	378.40 ± 0.75
(04)	NA	0.79 ± 1.45	3.06 ± 1.57	17.67 ± 4.99	36.51 ± 4.52	53.55 ± 2.13	Sat	380.15 ± 0.33
(05)	4.39 ± 0.37	6.83 ± 2.70	21.10 ± 1.89	45.09 ± 7.13	61.53 ± 0.91	67.26 ± 0.68	Sat	146.06 ± 6.08
(06)	3.75 ± 0.48	7.34 ± 1.20	20.15 ± 0.78	48.57 ± 8.84	68.46 ± 0.52	77.58 ± 14.56	Sat	125.02 ± 1.67
	1.5625	3.125 µg	6.25 µg	12.5 µg	25 µg	50 µg	100 µg	
BHT	34.66 ± 2.62	49.23 ± 0.77	61.29 ± 0.69	68.89 ± 0.26	70.02 ± 0.50	70.49 ± 0.55	71.13 ± 0.74	3.32 ± 0.18
BHA	25.99 ± 2.56	39.15 ± 0.88	54.16 ± 0.27	65.02 ± 1.26	70.19 ± 0.51	70.32 ± 0.65	70.60 ± 0.10	5.38 ± 0.06

CUPRAC Test

According to the results (Table 7) we notice that the extract 05 gave better results with $A_{0.5}$=88.25±3.27µg /ml but the extracts 02, 03, 04, 06 have low activity with high values of $A_{0.5}$ =229.44±3.85 µg/ml, 594.63±0.80 µg/ml, 112.11±6.27 µg/ml and 169.33±2.25 µg/mL, respectively.

Ayda Khadri et al., from Tunisia have evaluated antioxydant activity using DPPH where ethanol extract from the leaves of *R. montana* show the best results with IC_{50} =1.47±0.1 µg/ml.[18] Ines Ouerghemmi et al. found that spontaneous and cultivated *Ruta* leaf and flowers extracts both had higher scavenging activity than the stem extracts.[36] Mohammedi from Algeria evaluated the antioxidant activity of *Ruta montana* essential oils collected from 7 different places in Algeria.[33] He found that essential oils from the Djelfa and Msila presented the best antioxidant power using two tests: DPPH and Reducing Power with IC_{50}= 49.6±2.7 and 50.2 ± 3.3 mg/L, respectuvely. The antioxidant activity of essential oil of *Ruta Montana* from Morocco,[37] has a high antioxidant potential with IC_{50} = 548.27±4µg/ml

Alexandra T.Coibra et al., did a synthesis work on the genus *Ruta*. So she gathered all the antioxidant activity evaluation works around the world, where all the results claim that the extracts of the genus *Ruta* are rich in antioxidants which give them a very important antioxidant power.[38]

Mounira Meghem and Saliha Dahamna have evaluate in vitro, antioxydant activities of *Ruta Montana* from Setif (Algeria) by three methods: DPPH, Bleaching of β-carotene and chelation of ferrous iron with a higher antioxidant capacity in ethyl acetate extract (IC_{50}= 0.044±0.001 mg/ml).[39]

In the work of Taoufik Benali from Morocco,[40] the antioxidant power of the essential oil of *Ruta Montana* has been estimated with IC_{50} = 244.62 ± 0.34 µg/ml. While M. Merghem from Algeria (Setif) was able to evaluate the antioxidant activity of *Ruta Montana* extracts *in vivo*.[41]

Anti-cholinesterase activity (AchE)

Table 08 shows the results obtained by the anticholinestrase test where it is observed that only extract 04 gave a better activity (IC_{50}=159.64±0.49µg/ml) which is considered weak compared to activity of the standard used (IC_{50}=6.27±1.15µg/ml) and that the extracts 02 and 05 have IC_{50} greater than 200µg/ml.

Table 4: Absorbance in reducing power assay

Extracts	Absorbance in reducing power assay							
	3.125 µg	**6.25µg**	**12.5 µg**	**25 µg**	**50 µg**	**100 µg**	**200 µg**	**$A_{0.5}$ µg/mL**
(01)	NA	NA	NA	NA	NA	NA	NA	NA
(02)	0.06 ± 0.00	0.06 ± 0.01	0.07 ± 0.00	0.08 ± 0.01	0.10 ± 0.02	0.15 ± 0.01	0.20 ± 0.02	>200
(03)	NA	NA	0.05 ± 0.01	0.05 ± 0.00	0.06 ± 0.01	0.07 ± 0.00	0.15 ± 0.02	>200
(04)	0.05 ± 0.00	0.06 ± 0.00	0.09 ± 0.02	0.12 ± 0.03	0.20 ± 0.01	0.36 ± 0.03	0.43 ± 0.00	>200
(05)	0.05 ± 0.01	0.06 ± 0.01	0.09 ± 0.01	0.13 ± 0.02	0.26 ± 0.02	0.39 ± 0.07	0.59 ± 0.06	135.40 ± 4.67
(06)	0.05 ± 0.00	0.06 ± 0.00	0.07 ± 0.00	0.09 ± 0.03	0.20 ± 0.02	0.31 ± 0.03	0.42 ± 0.07	>200
Ascorbic acid	0.35 ± 0.05	0.46 ± 0.03	0.84 ± 0.12	0.93 ± 0.30	1.18 ± 0.34	1.37 ± 0.20	1.44 ± 0.21	6.77 ± 1.15
α-Tocopherol	0.11 ± 0.00	0.16 ± 0.00	0.21 ± 0.03	0.35 ± 0.03	0.73 ± 0.03	1.37 ± 0.08	1.81 ± 0.09	34.93 ± 2.38

Table 5: Absorbances in phenanthroline assay

Extracts	Absorbances in phenanthroline assay							
	3.125 µg	**6.25 µg**	**12.5 µg**	**25 µg**	**50 µg**	**100 µg**	**200 µg**	**$A_{0.5}$ µg/mL**
(01)	NA	NA	NA	NA	NA	NA	NA	NA
(02)	0.28 ± 0.01	0.28 ± 0.01	0.30 ± 0.01	0.29 ± 0.00	0.35 ± 0.01	0.39 ± 0.01	0.51 ± 0.02	181.44 ± 7.60
(03)	NA	NA	NA	0.26 ± 0.00	0.27 ± 0.00	0.30 ± 0.01	0.32 ± 0.00	>200
(04)	0.31 ± 0.02	0.31 ± 0.01	0.34 ± 0.01	0.40 ± 0.01	0.52 ± 0.02	0.65 ± 0.04	0.98 ± 0.08	46.21 ± 3.99
(05)	NA	0.37 ± 0.02	0.40 ± 0.02	0.45 ± 0.02	0.61 ± 0.03	0.79 ± 0.03	1.19 ± 0.08	35.06 ± 0.49
(06)	NA	0.34 ± 0.01	0.37 ± 0.01	0.38 ± 0.01	0.46 ± 0.01	0.62 ± 0.03	0.86 ± 0.01	60.40 ± 0.69
	0.78125µg	1.5625µg	3.125 µg	6.25 µg	12.5 µg	25 µg	50 µg	
BHA	0.49 ± 0.01	0.59 ± 0.01	0.73 ± 0.02	0.93 ± 0.01	1.25 ± 0.04	2.10 ± 0.05	4.89 ± 0.06	0.93 ± 0.07
BHT	0.47 ± 0.01	0.47 ± 0.01	0.53 ± 0.03	1.23 ± 0.02	1.84 ± 0.01	3.48 ± 0.03	4.84 ± 0.01	2.24 ± 0.17

Table 6: Absorbance in SNP

Extracts	Absorbance in SNP							
	6.25 µg	**12.5 µg**	**25 µg**	**50 µg**	**100 µg**	**200 µg**	**400 µg**	**$A_{0.5}$µg/mL**
(01)	NA	NA	NA	NA	NA	NA	NA	NA
(02)	0.07 ± 0.00	0.08 ± 0.01	0.1 ± 0.01	0.15 ± 0.03	0.15 ± 0.03	0.22 ± 0.06	0.29 ± 0.08	>400
(03)	0.06 ± 0.00	0.07 ± 0.01	0.09 ± 0.00	0.09 ± 0.03	0.11 ± 0.01	0.20 ± 0.02	0.41 ± 0.03	>400
(04)	0.08 ± 0.02	0.10 ± 0.01	0.12 ± 0.01	0.20 ± 0.00	0.28 ± 0.01	0.41 ± 0.01	0.57 ± 0.04	296.09 ± 1.02
(05)	0.06 ± 0.00	0.07 ± 0.00	0.09 ± 0.00	0.13 ± 0.01	0.24 ± 0.02	0.34 ± 0.04	0.50 ± 0.05	456.96 ± 2.78
(06)	0.05 ± 0.01	0.06 ± 0.00	0.08 ± 0.01	0.12 ± 0.00	0.19 ± 0.01	0.31 ± 0.01	0.50 ± 0.06	356.67 ± 0.52
	1.5625µg	3.125µg	6.25µg	12.5µg	25µg	50µg	100µg	
AC ascorbique	0.24 ± 0.00	0.32 ± 0.01	0.45 ± 0.01	0.75 ± 0.01	1.22 ± 0.02	1.90 ± 0.03	2.35 ± 0.01	7.14 ± 0.05
trolox	0.17 ± 0.00	0.20 ± 0.01	0.23 ± 0.00	0.30 ± 0.01	0.43 ± 0.01	0.64 ± 0.03	1.04 ± 0.01	34.17 ± 1.23

Ayda Khadri *et al*.[18] from Tunisia have evaluated antiacetylcholinesterase of *R. montana* and *R. chalepensis* and they found that the greatest inhibitory activity was showed by leaf ethanol extract of *R. chelepensis* (IC_{50}=12±1.1 µg/mL)

Antifungal activity

According to these results (Table 9 and Figure 1), it is found that the plant extract 04 gave better antifungal activity against the fungus (*Fusarium oxysperum f.sp. lycopersici* (FOL)) with a fungistatic effect which is translated into an inhibition rate of 11.91% at 1mg and 60.91% at 40mg; the rest of the extracts gave a moderate to weak fungistatic effect with an inhibition rate of 39.20% of the 05 extract at 40mg and 4.88% of the 06 extract at 40mg. Essential oil of *Ruta Montana* from Morocco,[37] showed strong antifungal activity.

GC-FID and GC-MS analysis

The chromatograms of the three extracts: essential oil (01), Soxhlet extract (02) and the hexane extract (03) are represented in Figures 2, 3 and 4 respectively.

65 compounds are identified in the three extracts, including 47 in the essential oil, with a common presence of: 2-nonanone (24.93%, 9.24% and 13.31% respectively), 2-undecanone (22.62%, 10.53% and 8.44% respectively), and the acetic acid, non-2-yl ester (14.15 %, 6.18% and 5.14% respectively) (Table 10).

A high presence of aliphatic alkanes was noted in extracts 02 and 03, varying from 56.89% in extract 02 to 47.19% in extract 03. The alkaloids were present more in the hexane extract (03) with dictamnine,6,7-dimethoxy with a rate of 8.39% in extract 02 and 0.93% in extract 03, Furocoumarine with 1.41% in extract 03, bergapten with 0.89% in extract 03 and Skimmianin with 0.74% in extract 03.

From *Ruta montana*, Driss Touati succeeded in isolating two known and 4 new alkaloids of the quinoline and 4 quinolone type.[9] Also, Kabouche isolated a new dicoumarinyl ether and two rare furocoumarins.[10] Eun-Tae Oh and her team identified Alkylquinolone alkaloid profiles in *Ruta Graveolens*.[11] For the composition of its essential oil, we can cite the work of N. Kambouche and his collaborators who were able to identify undecan-2-one (32.8%) and nonan-2-one (29.5%) as major compounds.[13] Djarri *et al*. were able to find that the major compound in the essential oil of *Ruta Montana* from Constantine was undecan-2-one with a percentage of 37.74%, followed by resorcinol (27.66%).[15]

Table 7: Absorbance sin CUPRAC assay

Extracts	Absorbance sin CUPRAC assay							
	12.5 µg	**25 µg**	**50 µg**	**100 µg**	**200 µg**	**400 µg**	**800 µg**	**$A_{0.50}$ (µg/mL)**
(01)	0.12 ± 0.1	0.13 ± 0.1	0.15 ± 0.01	0.16 ± 0.01	0.16 ± 0.00	0.23 ± 0.00	0.36 ± 0.05	>800
(02)	NA	0.16 ± 0.00	0.20 ± 0.00	0.30 ± 0.02	0.44 ± 0.03	0.80 ± 0.01	1.35 ± 0.13	229.44 ± 3.85
(03)	NA	0.14 ± 0.04	0.16 ± 0.02	0.18 ± 0.00	0.24 ± 0.11	0.35 ± 0.01	0.63 ± 0.03	594.63 ± 0.80
(04)	0.18 ± 0.02	0.21 ± 0,00	0.31 ± 0.00	0.49 ± 0.03	0.79 ± 0.02	1.34 ± 0.05	2.02 ± 0.05	112.11 ± 6.27
(05)	0.18 ± 0.01	0.28 ± 0.11	0.36 ± 0.01	0.53 ± 0.04	0.97 ± 0.02	1.59 ± 0.03	2.76 ± 0.03	88.25 ± 3.27
(06)	0.16 ± 0.01	0.19 ± 0.01	0.33 ± 0.10	0.39 ± 0.01	0.64 ± 0.01	1.09 ± 0.00	1.79 ± 0.09	169.33 ± 2.25
BHA	1.12 ± 0.05	1.95 ± 0.31	3.14 ± 0.46	3.58 ± 0.42	3.35 ± 0.20	3.77 ± 0.19	3.92 ± 0.13	5.35 ± 0.71
BHT	1.41 ± 0.03	2.22 ± 0.05	2.42 ± 0.02	2.50 ± 0.01	2.56 ± 0.05	2.86 ± 0.07	3.38 ± 0.13	8.97 ± 3.94

Table 8: Acetylcholinesterase inhibitory activity

Extracts	Acetylcholinesterase inhibitory activity							
	3.125 µg	**6.25 µg**	**12.5 µg**	**25 µg**	**50 µg**	**100 µg**	**200 µg**	**IC_{50} µg/mL**
Huileess (01)	NA	NA	NA	NA	NA	NA	NA	NA
Soxlet(02)	NA	NA	NA	NA	NA	NA	0.25 ± 9.83	>200
Hexane(03)	NA	NA	NA	NA	NA	NA	NA	NA
Chloroform(04)	NA	NA	NA	8.56 ± 8.11	27.37 ± 1.84	46.50 ± 1.53	51.36 ± 5.20	159.64 ± 0.49
AcEt(05)	NA	NA	NA	NA	NA	NA	18.98 ± 2.50	>200
BuOH(06)	NA	NA	NA	NA	NA	NA	NA	NA
Hexane(03)	NA	NA	NA	NA	NA	NA	NA	NA
Galantamine	35.93 ± 2,28	43.77 ± 0.00	68.50 ± 0.31	80.69 ± 0.41	85.78 ± 1.63	91.80 ± 0.20	94.77 ± 0.34	6.27 ± 1.15

Table 9: Antifungal activity

Extract	01		02		03		04		05		06	
Concentration of extract (mg/ml)	D(cm)	I%	D(cm)	I%	D(cm)	I%	D(cm)	I%	D(cm)	I%	D(cm)	I%
1	7.15	1.1787	6.825	3.4202	7.025	0.5900	6.2375	11.7339	6.6	6.6042	6.925	2,0051
2	7.283	3.0608	6.625	6.2504	6.7	5.1891	6.225	11.9108	6.333	10.3824	-	-
4	6.975	1.2976	6.575	6.9579	6.25	11.5570	5.575	21.1088	5.85	17.2173	6.975	1,2976
8	7.1	0.471	6.433	8.9674	6.033	14.6277	4.6667	33.9621	5.325	24.6465	6.825	3,4202
16	6.875	2.7127	6.233	11.7975	5.325	24.6465	3.8	46.2266	4.8833	30.9012	6.825	3,4202
40	5.75	25.08	6.2	19.218	4.933	35.7263	3	60.91	4.666	39.20	7.3	4,88

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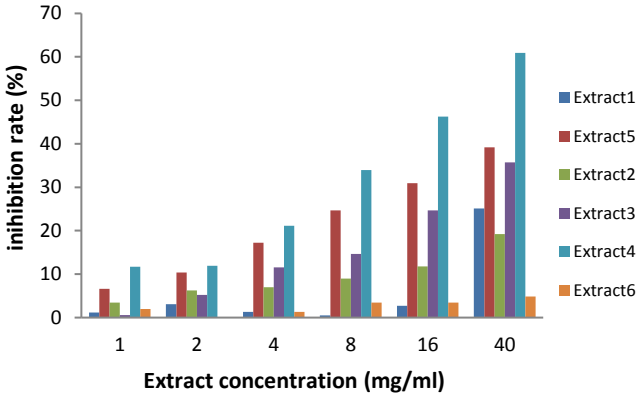

Figure 1: Antifungal activity of all extracts in different concentrations

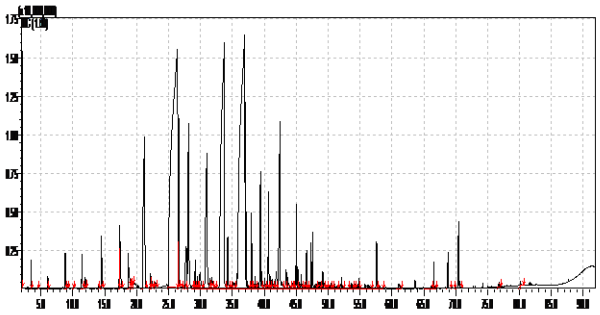

Figure 2: Essential oil extract (01) of *Ruta montana* GC-MS chromatogram

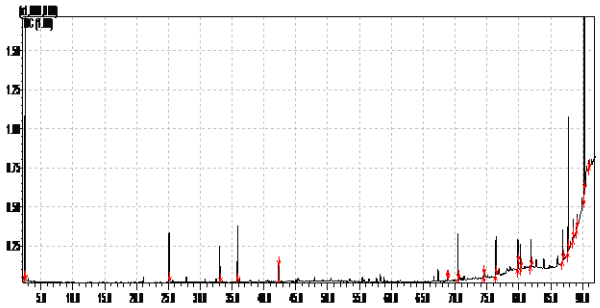

Figure 3: Soxhlet extract (02) of *Ruta montana* GC-MS chrommatogram

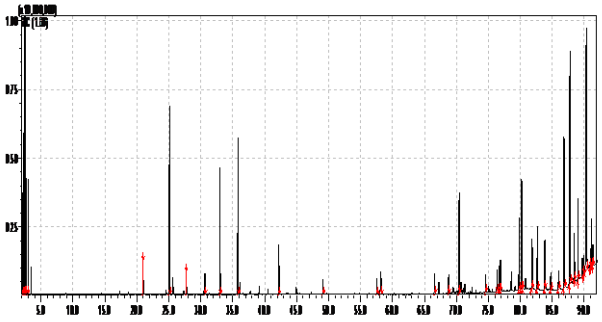

Figure 4: Hexane extract (03) of *Ruta montana* GC-MS chrommatogram

The work of Ines Hammami has identified a totally different composition in the essential oil of *Ruta montana* from Tunis with 1-butene as the major compound (38.33%).[12] While Mohammedi et al. were able to identify two groups with statistical analysis in essential oils from 7 régions of Algeria: the 2-undecanone group (it includes 2 regions) and a second group with a mixture of majority composition which is: 2-undecanone, 2-nonanone and 2- nonanol acetate.[33] Although this second group is the closest to our composition, but no analysis has identified 2-nonanone as the major compound, which makes our essential oil a bit special compared to the other oils studied previously. From Morocco, Taoufik Benali identifies 2-undecanone with a percentage of 63.97%.[40] Zineb Bennaoum identified the composition of 11 *Ruta*, always with 2-undecanone as the main compound.[42] Mohammed Barbouchi, analyzed the essential oils of *Ruta montana* from 4 regions of Morocco, 2-nonanone was only identified in two essential oils with rates of 1.77 and 8.64%, on the other hand 2-undecanone was present in the 4 essential oils with a rate that varies from 4.29 to 74.36%.[43]

Conclusion

In conclusion, the study on *Ruta Montana* extracts from Souk Ahras, Algeria, revealed significant biological activities, including antioxidant, antifungal, and anti-cholinesterase properties. These activities can be attributed to the presence of flavonoids and polyphenols in the plant extracts. The extracts also showed potential in inhibiting the growth of *Fusarium oxysperum f.sp lycopersici* (FOL), a fungus responsible for vascular wilt in tomatoes. Chemical analysis identified Acetic acid, non-2-yl ester, 2-Undecanone, and 2-Nonanone as major compounds, along with the presence of aliphatic alkanes. These findings highlight the potential of *Ruta Montana* as a valuable natural resource with various bioactive components and potential applications in pharmaceutical and agricultural industries.

Table 10: Composition of 3 extracts of *Ruta. Montana* by GC-MS

N°	Compound identidied	KI	% of essential oil (1)	% of Soxhlet extract (2)	% of Hexane Extract (3)
01	2-methyl pentane	570	-	-	1.57
02	3-methyl pentane	580	-	-	3.19
03	Acetic acid ethyl ester	608	-	-	1.58
04	Methylcyclopentane	661	-	-	4.63
05	Cyclohexane	675	-	-	1.58
06	Heptane	700	-	-	0.44
07	Ethylfuran	742	0.10	-	-
08	2-pentylthiol	758	0.02	-	-
09	Hexanal	801	0.09	-	-
10	2-hexenal E	814	0.41	-	-
11	2-Ethyl-5-methyltetrahydrofuran	835	0.06	-	-

12	Hexanol	860	0.02	-	-
13	Isopropyl 2-methylbutyrate	916	0.37	-	-
14	1R alpha pinene	948	0.55	-	-
15	sabinene	964	0.70	-	-
16	Beta pinene	966	0.50	-	-
17	Octanal	1007	0.02	-	-
18	Alpha limonene	1018	**4.44**	-	0.18
19	2-Heptyl acetate	1021	0.17	-	-
20	Gamma terpinene	1046	0.11	-	-
21	2-Nonanone	1052	**24.93**	**9.24**	**13.31**
22	2-Nonanol	1078	2.57	-	-
23	Mentha-2,8-dien-1-ol (trans, para)	1122	0.03	-	-
24	Pregeigeren	1145	0.90	-	Tr
25	Cyclohexene-5,6-diethenyl-1-methyl	1155	**3.40**	-	-
26	2-methoxy-3-isopropyl-(5 or 6) methyl pyrazine	1173	0.13	-	-
27	3-decanone	1180	0.30	-	-
28	Decanal	1181	0.13	-	-
29	Terpinen-4-ol	1182	0.34	-	-
30	2-decanone	1188	**3.82**	-	0.75
31	Decanal	1195	0.13	-	-
32	Octyl acetate	1203	0.23	-	-
33	Acetic acid, non-2-yl ester	1221	**14.15**	**6.18**	**5.14**
34	3-Undecanone	1251	0.47	-	-
35	2-Undecanone	1255	**22.62**	**10.53**	**8.44**
36	2-Undecanol	1275	0.63	-	-
37	Nonyl acetate	1282	0.02	-	-
38	Acetic acid, dec-2-yl ester	1332	0.18	-	-
39	3-dodecanone	1340	1.86	-	-
40	2-dodecanone	1350	1.18	-	-
41	2-dodecanol	1376	0.17	-	-
42	Bicyclo[5.2.0]nonane,2-methylene-4,8,8-trimethyl-4-vinyl	1393	0.32	-	-
43	2-undecanol, acetate	1425	**3.93**	**1.14**	**1.25**
44	3-tridecanone	1440	0.25	-	-
45	2-tridecanone	1449	1.09	-	-
46	Tridecanol, methylester	1488	0.23	-	-
47	2-dodecanol, acetate	1516	0.11	-	-
48	2,4-tridecandione	1572	0.17	-	-
49	4-(3,4-methylenedioxyphenyl)-2-butanone	1585	0.21	-	0.48
50	Tetradecanal	1605	0.07	-	-
51	Furocoumarine	1717	-	-	***1.41***
52	Bergapten	2065	-	-	**0.89**
53	phytol	2106	**0.29**	**Tr**	**0.65**
54	Skimmianin	2330	-	-	***0.74***
55	Dictamnine,6,7-dimethoxy	2470	-	***8.39***	***0.93***

56	pentacosane	2500	0.06	-	0.93
57	Palmitic acid, N-octyl ester	2578	-	-	-
58	heptacosane	2700	**0.05**	**3.17**	**3.87**
59	13-tricosen-1-ol	2759	-	-	2.63
60	Nonacosane	2900	-	-	2.16
61	triacontane	3000	-	-	0.88
62	hentriaconatne	3100	-	**22.02**	**16.10**
63	Alpha tocopherol	3149	-	-	1.70
64	dotriacontane	3200	-	-	2.51
65	tritriacontane	3300	-	**31.70**	**20.74**
	Total identified		92.53	98.37	98.69

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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