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Research Article

Analysis of the Chemical Compositions of the Alcoholic Extract and the Essential Oil of the Leaves of the Plant *Myrtus communis* from Blida – Algeria

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Abstract

The current research focuses on the study the chemical composition of the alcoholic extract and the essential oil the leaves of *myrtus communis* that was collected in the mountainous region in Blida, Algeria. Histological cuts have been made to reveal the presence of sub-epidermal secretory pockets at leaf level. The phyto-chemical screening of this plant revealed the presence of flavonoids, tannins of terpenes, free Quinones of anthocyanins, and reductive compounds. As for saponins, coumarins and alkaloids, they are absent in the leaves. The antioxidant potentilla of the alcoholic extract was revealed by the DPPH. The identification of the chemical compositions of the alcoholic extract s sheet were analyzed by HPLC showed 30 components representing 99.97% of the total extract. The main components were: genistein (29.48%), methyl Quercetin (16.56%), Kaempferol (13.26%), pinobanksin-3-acetate (9.18%), prenyl caffeine (6.89%). And 15 components of essential oil represent NT at 96.07% were identified by CPG/Ms, the majority compounds are α-pinene (21.07%), 1.8 cineole (27,01%), myrtenyl acetate (20,19%).

Keywords: Myrtus Communis; Alcoholic Extract; Essential Oil; HPLC; CPG/Ms

Introduction

The plant that is to be searched is the *myrtus communis* L. is a medicinal species belonging to the Myrtaceae family [1] contains 16 species that grow spontaneously in the regions Mediterranean but also in the Tropics (Algeria, Tunisia, Morocco, Turkey, France and Iran) and in Australia., it's a shrub plant. The leaves, flowers and berries of *myrtus communis* L. have been used for a long time for medicines, foods such as the aroma of meat and sauces, spices and cosmetics [2] this plant emits a pleasant odor when the leaves and flowers are crushed, mainly because of the essentials [3].

Myrtus communis L, registered in the European Pharmacopoeia [4] and commonly used for its anti-septic and antimicrobial antispasmodic properties, astringent and toning agents, as well as its anti-parasitic properties. Myrtle is also well known for its anti-genotoxic and antioxidant properties, in addition to its hem-agglutinating, anti-Hyperglycemic, Hypo-cholesterolemiant and anti-inflammatory [5].

As well as the leaves contain many compounds such as tannins, flavonoids such as quercetin, catechin, myricetin derivatives and volatile oils [6].

The objective of this work is to evaluate and determine the components of the ethanolic extract and essential oil of the leaves of myrtus communis of Blida, Algeria.

Material and Method

Plant material

The plant of *myrtus communis* L is formed by the leaves (whole, opposite) and the fruits that were harvested in the fresh State during the month of mid-June 2017 randomly. in the mountainous areas of chreaa (Figure 1), located in the region Southeast of Blida, Province located in the Central tell North of country table 1. *Myr-*

Figure 1: Harvesting area.

Region	Altitude	Latitude	Longitude	Bioclimatic floor	Characteristic
CheréaBlida	158 to 1627 m	36° 25′ 32”North	2° 52′ 36”East	Humid and sub-humid	High Mountain National Park

Table 1: Geographical coordinates of the harvesting site.

tus communis L were preserved dried and glued on a rigid support (Figure 2) identified by Pedro sànchez Gomez at the University of Murcia.

Figure 2: Dried Myrtle and glued to a rigid support.

Histological study of the plant structures secretory of essential oils of *Myrtus communis* L

The plant is subjected to microscopic examination by making anatomical cuts at the level of the leaves and stems, which are treated by the technique of double staining. The colouring is done on material fixed in alcohol at 70o (dead cell) the cuts have undergone the following treatments according to the Protocol borrowed from [7].

Analyses phytochimiques

The research of different chemical groups in the leaves of myrtus communis, was carried out according to the standard methods.

Preparation of extracts

By maceration

Extraction was carried out by exhaustion of the vegetable powder by maceration in the water/ethanol mixture (2/8). The raw extract obtained is subjected to a double filtration, then concentrated to the rotating evaporator and finally dried at room temperature. The recovered dry residue is weighed to determine its yield and kept cool, in a well-closed dark vial, to perform subsequent phytochemical tests and antioxidant activities.

By hydro-distillation

A biomass of 200g of plant leaves was subjected to a hydro distillation for 3 h, using a modified Clevenger-type apparatus. The essential oil collected by settling at the end of the distillation was dried on anhydrous sodium sulphate to remove residual water traces. The resulting gasoline was put into small opaque vials and stored at 4°C before use.

Anti-oxidant activity

Tested by 2.2-diphenylpicrylhydrazyl (DPPH)

This test was carried out following the free radical trapping method DPPH Mamadou [8]. Ten (10) concentrations of the extract were prepared in the appropriate solvent (distilled water and ethanol). 200 µl of each of the extract solutions (solvent only for white) were mixed with 3800 µl of a DPPH solution at 100 µM and placed in the dark. The kinetics of the reaction was followed during 2H, with measurement of absorbance at the spectrophotometer at 517nm every 15 minutes. And for the HE using was performed according to the Protocol described by Schlesier and his collaborators (Schlesier K., et al. 2002). The results are expressed as a percentage of inhibition of DPPH, this percentage is calculated according to the following formula.

The trapping rate of DPPH radicals was calculated according to the equation:

DPPH trapping rate (%) = (control absorbance – ABS extracted)/ ABS control x 100

The equilibrium time of the reaction for each of the extracts and their concentration of extract inhibiting 50% of the DPPH˙ radicals (IC^{50}) were determined. Ascorbic acid was used as a reference standard.

Determination of phenolic compounds by HPLC/SM

In order to identify the different bioactive organic components present in the leaves of the plant *Myrtus communis* L by the technique of analysis and separation HPLC, several samples of leaves were transported from the laboratory "Bio-toxicology, pharmacognosy and plant biological enhancement", Tahar-Moulay University of Saida, Algeria to the laboratory "pharmaceutical chemistry", Faculty of pharmacy, free University of Brussels, Brussels 1050, Belgium, Belgium. Analyses of the leaf breathalysis were performed on a liquid phase chromatograph coupled by mass spectrophotometry LC/MS-MS (HPLC). The mass spectra were obtained with a QTOF6520 (Agilent, Palo Alto, CA, USA) using a column Zorbax Eclipse XDB C18 fast resolution HT 4.6 x 50 mm, 1.8 UM, in positive ESI mode, with a flow rate of 0.4 mL/min, with 10 mM ammonium acetate phas e mobile (solution A): CH3OH (solution B) in a gradient mode as follows (B% hour): (10%, 0min) (95%, 10min), (10%, 15min), (VCAP 3500 eV; Source T°, 350°C; fragliar, 110V; skimmer, 65V).

Analysis of essential oil by CPG/Ms

GC-MS analysis of essential oils was performed using a Fisons 8000 series gas chromatograph (model 8060) coupled with a Fisons® 800 quadripole mass spectrometer (Fisons instruments, Manchester, UK). equipped with an injector in split mode, a capillary column DB-wax (30 m ≤ 0.25 mm, film thickness = 0.25 LM, J and W). The ionization mode is the electronic impact with an ionization energy of 70 eV. The operating conditions are as follows: solvent: ethyl acetate injection temperature 220˚C, injection volume 1 µl, flow 1.0 ml/min, with oven temperature programming from 40˚C to 250˚C, at the rate DE4˚C/min and maintained at 2500C for 5 min, gas vector: helium. The coupling with the Polaris Q MS mass spectrometer is done with an interface temperature of 300 ˚C. The identification of the different constituents is carried out by comparing the mass spectra obtained with those of the computerized data bank Wiley 275. L and literature [9] confirmations are obtained by determining the retention indices and comparison with literature data [10].

Results

Study of the secretory structures of essential oils

Observation of the anatomical sections of the leaves and stems have been carried out by optical microscope, which has been allowed to visualize the different tissues and localization of the secretory pockets of the essential oils of the plant *myrtus communis.* The results of experimental research on leaves and stems, we have revealed the presence of subepidermal secretory pockets at the leaf level that are the synthesis role of essential oil (Figure 3,4).

Figure 3: leaf-level Anatomy cut (*myrtus communis*)

NP: the main rib (blue arrow); NS: secondary rib; PS: secretory pocket. Pp: Palisade parenchyma; PL: lacunous parenchyma.

Phytochimique test

Phytochemical tests consist of detecting the different families of secondary metabolites existing in the studied part of the plant by qualitative characterization reactions. These reactions are based on precipitation or staining phenomena by reagents spe

Figure 4: Anatomical cut of myrtus communis stem level

ER: residual epidermis. PM: Medullary parenchyma. PC: cortical parenchyma.

Phytochemical tests consist of detecting the different families of secondary metabolites existing in the studied part of the plant by qualitative characterization reactions. These reactions are based on precipitation or staining phenomena by reagents specific to each family of compounds. The phytochemical tests carried out on the extract of Myrte leaves revealed a strong presence of terpanoide, flavonoids, tannins, free Quinones and reductive compounds in the extracts while the coummarins, anthocyanins, saponosides, sterl and steroids are absent, concerning anthraquinones and alkaloids their presence variables have been revealed in the leaves of *myrtus communis* (table 2).

Anti-oxidant activity

The anti-oxidant activity of the extract was evaluated by the dpph method and the percentage of trapped radicals was expressed as a function of the extract concentration. In this test Ascorbic acid was used as a standard, the results obtained (percentage of inhibions I%) are represented in the calibration curve (Figure 5) that it showed anti-oxidant activity with the linear regression formula: y =6,445ln (x) + 77,68; R2 = 0.937.

Terpnoide	Alkaloids		Flavonoids	Coumarins	Saponosides	Tannins	Steroid and steroid	Reducing compounds	Free Quinones	Anthraquinones	Anthocyanes
	Reagent M	Reagent D									
+++	+	+	+++	-	-	+++	-	+++	+++	++	-
-= Absent; + = Present; + + = moderately present; + + + = strongly present; reactive M = Mayer; Reagent D = Dragdroff											

Table 2: Phytochemical screening of leaf extracts *Myrtus communis* L.

According to the illustrated curve of the anti-oxidant activity by dpph of the hydro-alcoholic extract, there is a proportional increase in the percentage inhibition in function of the concentrations of the extract, have allowed the curve to be obtained with an R_{two} other ranging from 0937.

The extract of the leaves of the species *myrtuscommunis* l showed a more important reductive power by at a concentration of 25mg/ml, it achieves a good inhibitory capacity with IC_{50}=0.014mg/ml (14µg/ml) found in the 15min equilibrium time.

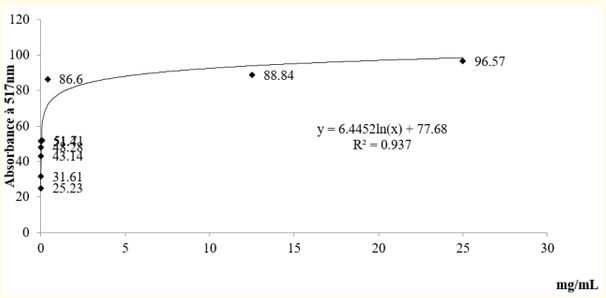

Figure 5: The concentration of the extract of the leaves *myrtus communis* in mg/ml at 517nm.

Analyze HPLC

The results of the HPLC – SM analysis is the alcoholic extract of the leaves of the plant *myrtus communis* L are presented in tables 3 and 4.

n	Compositions	The formula	Rt	%
01	Acacetin	C16 H12 O5	11.786	2.88
02	naringinine	C15 H12 O5	11.833	2.12
03	Tectochrysin	C16 H12 O4	11.85	3.39
04	catéchine	C15 H14 O6	11.864	0.03
05	methyl quercétine	C16 H12 O7	11.876	16.56
06	Pinocembrin	C15 H12 O4	11.896	2.07
07	quercétine	C15 H10 O7	11.933	3.61
08	sucrose	C12 H22 O11	11.958	0.01
09	Bis-methylated quercétine	C17 H14 O7	11.966	3.66
10	Acide Ferulic methyl ester	C11 H12 O4	11.978	0.01
11	Pinobanksin-3-acetate	C17 H14 O6	12.004	9.18
12	genistéine	C15 H10 O5	12.015	29.48
13	Methoxy-pinobanksin	C16 H14 O5	12.052	1.43
14	chrysine	C15 H10 O4	12.149	0.31
15	cinnamic acid	C9 H8 O2	12.198	0.25
16	caffic acid	C9 H8 O4	12.237	3.53
17	Prenyl caffeate	C14 H16 O4	12.242	6.89
18	Coumaric acid methyl ester	C10 H10 O3	12.251	0.12
19	Kaempferol	C15 H10 O6	12.284	13.26
20	Resveratrol	C14 H12 O3	12.42	0.07
21	Pinobanksin-3-propionate	C18 H16 O6	12.553	0.04
22	coumaric acid	C9 H8 O3	12.904	0.01
23	Pinostrobin	C16 H14 O4	13.21	0.57
24	Totarol	C20 H30 O	13.269	0.09
25	Isoprenyl coumarate	C14 H16 O3	13.417	0.01
26	Isoprenyl ferulate	C15 H18 O4	13.598	0.04
27	Pimariic acid	C20 H30 O2	13.97	0.12
28	3-Hydroxy-4-methoxycinnamic acid	C10 H10 O4	14.367	0.03
29	Tyrosol	C8 H10 O2	14.429	0.12
30	Hexadecanoic acid	C16 H32 O2	14.516	0.08
	Total identified			99,97

Table 3: Chemical composition of leaf extract *myrtus communis L*.

The constituents	%
Phenolic acid	3.94
Flavonoids	
• Flavonol	77.22
• Flavone	6.58
• Flavanol	0.03
• Flavanone	4.76
Total	88.59
Terpène	0.25
Stilbène	0.07
other	0.21

Table 4: Classification of leaf constituents *Myrtus communis.*

Thirty compounds were identified in the ethanolic extract, representing 99.97% of the total extract. The latter was dominated by a quantity of flavonoid (88.59%), phenolic acid (3.94%), and components that were marked by a small amount are terpenes (0.25%) and stilbene (0.07%). The main constituents of the extract were revealed as genistein (29.48%), methyl quercetin (16.56%), Kaempferol (13.26%), Pinobanksin-3-acetate (9.18%), Prenyl caffeate (6.89%), bis-methylated quercetin (3.66%), quercetin (3.61%), acid cafique (3.53%), Tectochrysin (3.39%), Acacetin (2.88%), naringinine (2.12%), Pinocembrin (2.07%) and the other compounds were presented by a small amount. In General, the composition of the extract of *myrtus communis* l, was considered to be a rich source of flavonoid exactly flavonool.

However, the method of using the ethanol solvent confirmed the presence of the component.

Chemical composition of *Myrtus communis* essential oil by CPG/MS

Analysis of the essential oil of Myrtus communis, allowed the identification of 96.07% of the constituents. The Myrtle has the

highest just what of the order 1.8-cinèole 27.01%, α-pinene 21.07%, myrtenyl acetate 20.19%, limonene 13.70% (Figure 6). Et other compounds are also present with an average rate such as α-Terpinol 4.73%, linalool 3.84%, 2-methyl butyrate of isobutyl 1.66%, methyl eugenl 1.22% and the other compounds are found in small quantities (table 4).

Figure 6: Profile chromatography of the leaves *myrtus communis* analyzed at 280 nm.

Our results are in agreement with those satrani., *et al.* 2006, [11] revealed the richness of the Myrtle of Morocco and Serbia of the α-pinene, 1.8- cineole and amyrtenyl ketate.

No	Compounds	Min retention time	Concentration %
1	A-Pinène	14,940	21,070
2	B-Pinène	15,220	0,410
3	Limonene	16,150	13,700
4	2-methyl isobutyl butyrate	17,060	1,660
5	1,8-cinèole	17,420	27,010
6	Linalool	17,900	3,840
7	A-Terpinol	18,480	4,730
8	Myrtènol	20,660	0,210
9	Myrtenyl acetate	23,350	20,190
10	Geranyle acetate	26,120	0,880
11	Methyl eugénol	27,450	1,220
12	P-Cymène	28,610	0,150
13	Linalyl acetate acetate	29,150	0,310
14	humulène	30,170	0,050
15	caryophyllene oxide	30,410	0,640
Total identified Number identified: 15			96,070

Table 5: Determination by CPG/MS of the compounds of the essential oil of *Myrtus communis.*

Component class	Percentage of components identified
* Terpenic oxides	27,10%
* Monoterpènes	45,33%
* Monoterpenic alcohols	21,38%
* Sesquiterpènes	0,69%

Table 6: Different classes of compounds identified in the essential oil of the *myrtus communis* leaves.

Discussion

Medicinal plants are characterized by the qualitative and quantitative richness of polyphenols. Currently, researchers are looking for active principles of plant origin, associated with lengthening of life expectancy and health protection. Many epidemiological studies have shown that the consumption of extracts of the leaves *myrtus communis* allowed to fight against hyperglycemia, hypercholesterolemia and cardiovascular disease. To know these active molecules, one has to go through physicochemical analysis methods.

The leaves of *myrtus communis* are characterized by a low yield of essential oil (EH) about 0.15% (w/w) and this differs depending on the harvest season [12]. Phytochemical screening of Myrtle leaves revealed a strong presence of terpanoides, flavonoids, tannins, free Quinonessand reductive compounds. Our results are consistent with those of [6,13,14]. Moreover, [15] in addition to these metabolites, the presence of sterl and the total absence of AlcaláOIDs, Reductor compounds which is contrary to our results.

The realization of antioxidant activity (AOX) on the alcoholic extract and not the essential oil because of low yield it was obtained. The percentage of DPPH trapped was expressed in terms of the concentration of alcoholic extracts of the Myrtle leaves. The activity of the alcoholic extract (IC_{50} = 14 µg/ml) can be explained by its high content of Polyphenolic compounds. And in particular its richness in flavonoids known for their antioxidant property which accumulates during the vegetative cycle of the Myrtle plant [5]. Numerous studies have been reported on the species *Myrtles communis*, among which the ones produced on the leaves are reported by the work of [13] which showed that the IC_{50} = 8 µg/ml is close to our extracts. (Gardeli., *et al*. 2008) is comprised of IC_{50} between 0.0095 and 0.017 mg/ml these same authors have also shown that the extracts of *M. communis* are harvested in summer are the most antioxidant (Chabert, 2013). The main secondary metabolites of *M. communis* are polyphenols and essential oils. Analyses by HPLC and CPG/MS of the species of *Myrtus communis* were reported to be very rich in flavonoid and monoterpene hydrocarbon. According to [13] has been revealed 14 known components and other unknown bioactive in the methanolic extract of Myrtle from Tunisia such ashydrolysable tannins (gallotannins) in leaves (79.39%), flavonoids (61.38%) and catechin (36.91%), the latter one found in our results the presence of very low catechin (0.03%), and the total absence of hydrolyable tannins. And the predominance of phenolic compounds is consistent with the previous results reported by [16]

the presence of very small amounts of phenolics in the ethanolic extract of Myrtle from Italy except the catechins revealed a very small amount. And for the essential oil our results are in agreement with those [11] revealed the richness of the Myrtle of Morocco and Serbia of α-pinene, 1.8-cinèole and myrtenyl acetate. the compositions of the essential oil and the extract is quite variable according to the geographical region of production, the harvesting season and the duration of distillation and extraction by solvent [14].

Conclusion

The importance of used quantitative and qualitative analysis of phenoliq componentsin Myrtle leaves to study and analyse the therapeutic effects of the Te-plan. These results encourage further and more thorough studies on the pH composition of the enolic plant extracts and the evaluation of the antioxidant activity of each compound separately. Some phenolic compounds remain to be identified and additional biological tests should be performed to compare and classify their various properties whether they are beneficial or not. And chooses the extraction method that gives a good amount of active principles that play a role a therapeutic effect.

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