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The Evaluation of Fatty Acids And Other Compounds Extracted From *Pulicaria Crispa* (Forssk.) Oliv. Regading Some Pharmaceutical Effects

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Abstract

In conventional medicine, the plant *Pulicaria crispa* is used for colds, cough, colic, excessive sweating, and carminative disorders. Our study aimed to evaluate the biological properties of extracts with different polarities, specifically chloroform ($CHCl_3$), ethyl acetate (EtOAc), and n-butanol (n-BuOH) extracts derived from Pulicaria crispa (Forssk.) Oliv (P. crispa). The chemical profile of the CHCl3 was determined by Gas chromatography coupled to mass spectrometry (GC-MS), and the biological activities of P. crispa extracts were evaluated regarding antioxidant, anti glycation and anti acetylcholinesterase effects. Our findings represent the first record about the CHCl3 extract that showed the presence of 35 different compounds with three major ones, we name: 2,5-Dihydroxynaphthalene-1,4-dione, 14-methyl Pentadecanoic acid methyl ester and Hexadecane with percentage area of 16.19%, 12.37% and 8.99%, respectively. Furthermore, the biological investigation demonstrated that P. crispa extracts possess significant antioxidant properties and, for the first time, revealed their anti-acetylcholinesterase and anti-glycation activities. Notably, the EtOAc extract exhibited the highest antiglycation potency, surpassing even the standard reference compound. Authors claimed that further surveys are required to isolate major components and conduct *in vivo* and clinical trials to validate their potential as therapeutic agents.

Keywords: acetylcholinesterase inhibition; anti-glycation; antioxidant; GC-MS analysis; *Pulicaria crispa*

Introduction

According to World Health Organization (WHO) statistics, around 80% of African populations rely on traditional medicine for their primary health care needs. Africa, recognized for its immense biological diversity and regarded as the cradle of humanity, benefits from a tropical and subtropical climate that supports a vast array of plant species approximately 40,000 to 45,000 in total, with 5,000 of these being medicinal. African herbal medicine is one of the oldest and most diverse medical systems in the world, with healing practices deeply influenced by the cultural and regional beliefs of various African countries. Algeria, the largest country in Africa, possesses a rich botanical heritage with over 3,139 plant species [1]. The Algerian Sahara encompasses a wide variety of plant species that have been extensively studied in phytochemical researches, revealing remarkable biological and chemical richness. These findings have significantly advanced the field. Southern Algeria is home to four Pulicaria species [2], [3], [4] one of which is *Pulicaria crispa* (Forssk.) Oliv. , known locally as "shewihyah." this plant has various medicinal uses, including. treating colds, colon pain, and excessive sweating; and as a carminative [5]. Furthermore, it has applications in treating inflammation, hemorrhoids, and even vaginal cancer [6], this has been confirmed by numerous biological studies [7], [8], [9]. *Pulicaria crispa* (Forssk.) Oliv., synonym Francoeuria crispa (Forssk.) Cass., is an annual herb or perennial sub-shrub belonging to the family Asteraceae (Compositae). Form perennial herb, ascending with a hemispherical appearance, 12-75 cm high, densely branched from the base with stems that are white woolly tomentose or occasionally nearly glabrous. Its leaves are simple, sessile, somewhat amplexicaul, narrowly linear, acute to obtuse, denticulate, obovate-oblong to linear lanceolate, 0.5-1.5 $\times$ 0.5-0.6 cm, tomentose to nearly solitary, terminal, 0.3-0.6 cm across. Furthermore, its flowers are yellow disc florets (inflorescence), radiate, 0.5-1 cm across, aromatic, achenes glabrous, pappus uniseriate, with a few short bristles [10].

The current study aimed to uncover additional biological value for P. crispa by evaluating the inhibition potential of its various extracts against advanced glycation end products (AGEs), which are formed through non-enzymatic Maillard reactions. The accumulation of AGEs in the body is linked to numerous complications associated with diabetes, as hyperglycemia enhances protein glycation. AGEs also play a role in the pathophysiology of Alzheimer's disease, arthritis, and aging. [11]. Therefore, compounds that can inhibit AGE formation or break them down may hold potential as drugs, dietary supplements, or bioactive additives. To strengthen our research, we also conducted *in vitro* studies on the anti-acetylcholinesterase and antioxidant activities of different P. crispa extracts using several methods. Additionally, we identified the fatty acid composition of the extracts, complementing previous findings on other metabolite categories such as phenolics and flavonoids [12], [13].

Materials and methods

Plant material and preparation of extracts

P. crispa aerial parts were collected in May 2020 from southern Algeria, the region of Touggourt, during the flowering season. Professor Youcef Haliss (Scientific and Technical Laboratory for Arid Regions of Touggourt CRSTRA) determined the plant's botanical identity. The aerial portions were dehydrated and ground into a powder. The powdered plant material (500 g) was extracted three times overnight at room temperature with 70% (V/V) aqueous/ methanol. On a rotary evaporator, the extracts were mixed, concentrated under reduced pressure, and dissolved in distilled water (500 mL). The resultant solution was extracted sequentially with three portions of chloromethane (CHCl3), ethyl acetate, and n-butanol (300 mL /each). To obtain chloromethane (CHCl3), ethyl acetate (EtOAc) and n-butanol (n-BuOH) extracts, the organic phases were dried with sodium sulfate (Na_2SO_4), filtered, and concentrated in vacuum at room temperature.

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis was performed on Agilent technologies GC 17A gas chromatography equipped with a gross-linked HP 5MS column (30 m × 0.25 mm, film thickness 0.25 µm). The oven temperature was programmed as isothermal at 60 °C for 8 min, and the flow rate of helium gas was 0.5 mL/min helium. GC-MS was accomplished using an HP Agilent technology 6800 plus mass selective detector. The operating conditions were the same as for the analytical GC. The MS operating parameters were as follows ionization potential, 70 eV; ionization current, 2 A°; ion source temperature, 280 °C; resolution, 1000 scan time, 5 s; scan mass range, 34–450 u; spit ratio, 50:1; injected volume, 1.0 µL. The following temperature program was applied 40 °C for 8 min then 150 °C for 5 min at a rate of 5 °C/min to 260 °C for 15 min at a rate of 5 °C/min. The identification of compounds was based on their retention times in comparison with matching peaks available in the NIST and Wiley mass spectral libraries, as well as by comparing the fragmentation pattern of the mass spectra and their retention indices with those reported in the literature by the NIST Standard Reference Database (Version 2.4).

Inhibition of AGEs formation

The search for inhibitors of AGEs formation has gained significant attention due to their potential to mitigate the risks associated with AGEs-related disorders, as previously discussed. In

this study, the inhibitory effects of P. crispa extracts were tested using a bovine serum albumin (BSA)/ribose system against pentosidine formation. Briefly the protocol of inhibition of AGE formation was conducted as follow: the extracts to test (1µg to 1mg) in a phosphate buffer 50 mM pH 7.4 (NaN_3 0.02%), BSA (10 mg/mL) with D-ribose (0.5 M), were incubated in 96-well microtiter plates at 37 °C for 24 h before the measurement of the AGEs fluorescence. The fluorescence resulting from the previous mixture without the presence of D-ribose and under identical circumstances was removed in order to account the intrinsic fluorescence of the tested samples (blank). Negative control (-) i.e., 100% inhibition of AGEs formation, consisted of wells only with BSA. Positive control (+) i.e., no inhibition of AGEs formation consisted of wells containing BSA (10 mg/mL) and D-ribose (0.5 M) [11],[14],[15]. Results are expressed as 50% inhibition concentration (IC50) values and the inhibition % (I %) was calculated using the equation (1) :

$$I\% = \frac{Fluorescence\ intensity\ (sample) - Fluorescence\ intensity\ (blank\ )}{Fluorecsence\ intensity\ (+\ control) - Fluorescence\ intensity\ (-\ control)} \quad (1)$$

Determination of acetylcholinesterase inhibitory activity

Acetylcholinesterase (AChE) inhibitory activity was measured by slightly modifying [16],[17]. The reaction mixture included 20 µL of AChE solution, 10 µL of extract at various concentrations, and 150 µL of 100 mM sodium phosphate buffer (pH 8.0). Following a 15 min incubation at 25°C, 10 µL of 5,5′-dithiobis-(2-nitrobenzoic acid (DTNB) (0.5 mM) and 10 µL of acetylthiocholine iodide (0.71 mM) were added to the mixture. At 412 nm, the absorbance was measured after 15 min. Galantamine was used as a positive reference compound. The results were given as IC50 value (µg/mL) corresponding to the concentration showing 50% inhibition.

Determination of antioxidant activity

DPPH free radical scavenging assay

Modifications to previous approaches [18],[19] were made to assess the antioxidant activity by DPPH free radical assay. 160 µL of DPPH solution 0.1 mM (methanol) was mixed with 40 µL of the sample (extracts, pure compounds, and standards) at varying concentrations. After 30 min of incubation at 37 °C, the microplate reader was used to measure the absorbance of each mixture at 517 nm. As standards, the activity of butyl hydroxyl toluene (BHT) and butyl hydroxy anisole

(BHA) was examined. The formula (2) was used to determine the inhibition percentage of free radical scavenging.

$$\% = \frac{A0 - A1}{A0} \times 100 \quad (2)$$

Where A0 and A1 was the absorbance of the control and the absorbance of the extracts, respectively.

Metal chelating activity assay

The metal chelating activity assay was assessed according the following procedure, 2 ml of sodium acetate buffer (0.1 M at pH 4.9) and 50 µL iron (II) chloride (2 mM) were added to 0.5 ml the samples. 0.2 ml of ferrozine (5 mM) was added after a 30 min at room temperature incubation. At 562 nm, the absorbance was measured after 30 min. Standard metal chelator ethylenediaminetetraacetic acid (EDTA) was utilized in the experiment, while distilled water served as negative control [20].

The inhibition percentage of "ferrozine – Fe2+" complex formation was calculated as indicated formula (2), where A0 and A1 was the absorbance of the control and the absorbance of the extract/EDTA, respectively. Using linear regression, we determined the concentration of Fe2+ in the sample needed to chelate 50% of ions (IC50)

Reducing power

The reducing power was measured with slight modifications [21]. In brief, 50 µL of potassium ferricyanide (10 mg/mL) and 40 µL of phosphate buffer (0.2 M, pH 6.6) were added to a 10 µL of the sample solution at varying concentrations. The resulting mixture was incubated at 50 C° for 20 min. The solution was then diluted with 40 µl of distilled water after being mixed with 50 µL of a trichloroacetic acid solution (100 mg/mL) and 10 µL of a ferric chloride solution (1.0 g/L). A 96-well microplate reader was used to measure the absorbance at 700 nm. The antioxidants which were used as standards in this test are ascorbic acid, tannic acid, alpha-tocopherol, butyric acid, and para-hydroxybenzoic acid. The concentration giving an absorbance equal to 0.5 was measured and used to report the findings as A0.5 (µg/mL).

Superoxide anion

An adjusted colorimetric method was used [22] to determine the O2•- scavenging activity. To produce O2•-, we dissolved 20 mg of NaOH in 100 ml of DMSO. The O2•- was mixed with 40 uL

of extract at concentrations ranging from 6.25 µg/ml to 200 µg/ml, and then 30 uL of NBT (1 µg/ml in distilled water) was added. The ascorbic acid was used as positive control and the methanol as negative control. Immediately, following an incubation of 10 min at room temperature, the absorbance was measured at 560 nm and the proportion of O2•- inhibition was calculated using the equation (3):

$$I\% = \frac{A1 - A2}{A2} \times 100 \quad (3)$$

Where A1 and A2 are respectively absorbance of negative control and sample.

Statistical analysis

All the experimental results are mentioned as a mean ± standard deviation of three trials except for AGEs inhibition assay. The IC50 and A0.50 values were calculated by linear regression analysis, and variance analysis was performed by ANOVA using XLSTAT. Significant differences between means were determined by Tukey test, and p values <0.05 were considered as significant.

Results and Discussion

GC-MS analysis

The herbal formulations are developed, updated, and checked for quality in large part by analysing and extracting plant material. In light of this, the purpose of the current study was to identify the bioactive chemicals specifically fatty acids, that are contained in the CHCl3 extract of P. crispa by the use of GC-MS technique. The identified compounds in the CHCl3 extract of P. crispa are listed in the order of their column elution time (Table 1), the chromatogram and the chemical structure of six major components are presented in Figure 1 and 2 respectively.

Table 1: Chemical composition of P. crispa identified by the GC-MS analyses.

N°	Compound	Chemical Formula	RI	Area (%)
1	Hexanoic acid, propyl ester	$C_9H_{18}O_2$	10.8455	0.1461
2	1,5-Dimethyl-2-pyrrolecarbonitrile	$C_7H_8N_2$	11.4928	0.0318
3	Phenylethyl Alcohol	$C_8H_{10}O$	13.2383	0.1437
4	Benzeneacetic acid, methyl ester	$C_9H_{10}O_2$	14.2201	0.6107

5	Naphthalene	$C_{10}H_8$	14.3947	0.1373
6	Benzaldehyde, 3,4-dimethyl	$C_9H_{10}O$	14.8747	0.0594
7	Octadecanoic acid, 3-hydroxy-, methyl ester	$C_{19}H_{38}O_3$	15.4129	0.1347
8	Decanoic acid, methyl ester	$C_{11}H_{22}O_2$	16.3001	0.1040
9	3-Methyl-4-methylene-2-hexanone	$C_8H_{14}O$	16.5838	0.1687
10	2-Cyclopenten-1-one, 3-methyl-2-(2-pentenyl)-, (Z)	$C_{11}H_{16}O$	17.5002	0.3044
11	1,2-Cyclohexanediol, 4-methyl-1-(1-methylethyl)-, (1 α,2 α ,4α)	$C_{10}H_{20}O_2$	17.9729	0.1541
12	Undecanoic acid, 10-methyl, methyl ester	$C_{12}H_{22}O_2$	18.9256	0.8701
13	Dimethyl azelate	$C_{11}H_{20}O_4$	19.2456	1.0518
14	Hexadecane	$C_{16}H_{34}$	19.8565	8.9983
15	Undecane, 3,6-dimethyl	$C_{13}H_{28}$	21.1002	0.7227
16	Tridecanoic acid, 12-methyl-, methyl ester	$C_{15}H_{30}O_2$	21.2602	0.6882
17	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O$	22.5475	0.2897
18	4-(2,4-Dimethylcyclohex-3-enyl) but-3-en-2-one	$C_{12}H_{18}O$	22.7511	0.3641
19	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	23.4057	12.3700
20	n-Hexadecanoic acid	$C_{16}H_{32}O$	23.8348	3.1194
21	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	24.1911	1.0177
22	6,8-Nonadien-2-one, 6-methyl-5-(1-methylethylidene)	$C_{13}H_{20}O$	24.1911	0.8796
23	Hexadecanoic acid, 14-methyl-, methyl ester	$C_{18}H_{36}O_2$	24.3439	1.5922
24	7-Octadecenoic acid, methyl ester	$C_{19}H_{36}O$	25.0929	5.1305
25	Octadecanoic acid, methyl ester	$C_{19}H_{34}O$	25.2893	4.6922
26	2,6,6-tetramethyl,1-Cyclohexene-1-butanal	$C_{14}H_{24}O$	25.4493	1.4903
27	2,5-Dihydroxynaphthalene-1,4-dione	$C_{10}H_6O_4$	26.0093	16.1963
28	Eicosanoic acid, methyl ester	$C_{21}H_{42}O$	27.0493	0.7584
29	Tetratriacontane	$C_{34}H_{70}$	27.2675	0.9795
30	2,4-Dimethylbenzo[h]quinoline	$C_{15}H_{13}N$	29.5803	0.7136
31	2-ethylacridine	$C_{15}H_{13}N$	30.1694	1.4657
32	4-dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene) tyramine	$C_{16}H_{14}N_2O_4$	30.6930	2.3972
33	1H-Indole, 1-methyl-2-phenyl	$C_{15}H_{13}N$	31.4494	0.5679
34	beta-Amyrin	$C_{30}H_{50}O$	32.1476	3.2681
35	Taraxasterol	$C_{30}H_{50}O$	34.0094	4.1908
36	Olean-12-EN-3-alpha-yl acetate	$C_{32}H_{52}O_2$	36.4676	1.3300
37	Podocarp-13-en-12-ol	$C_{17}H_{28}O$	26.7220	2.8388

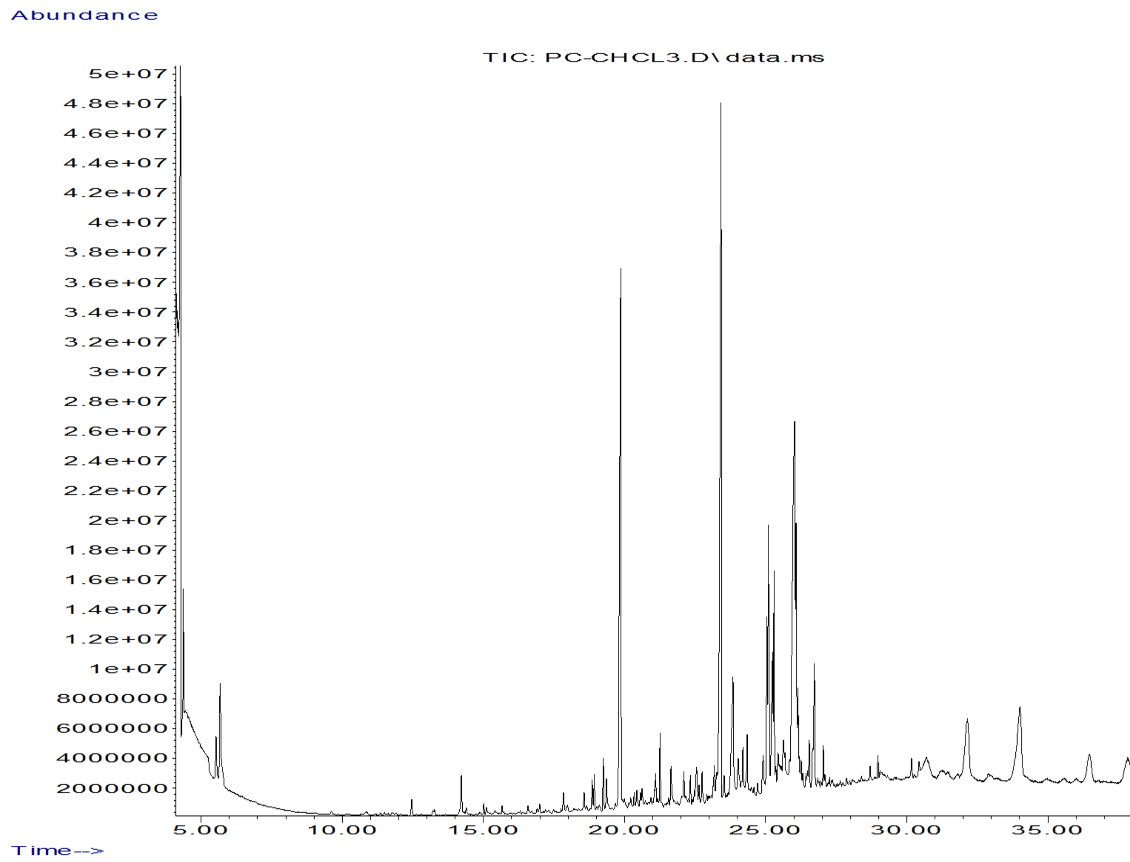

Figure 1: GC-MS chromatogram of the chloroform extract of P. Crispa.

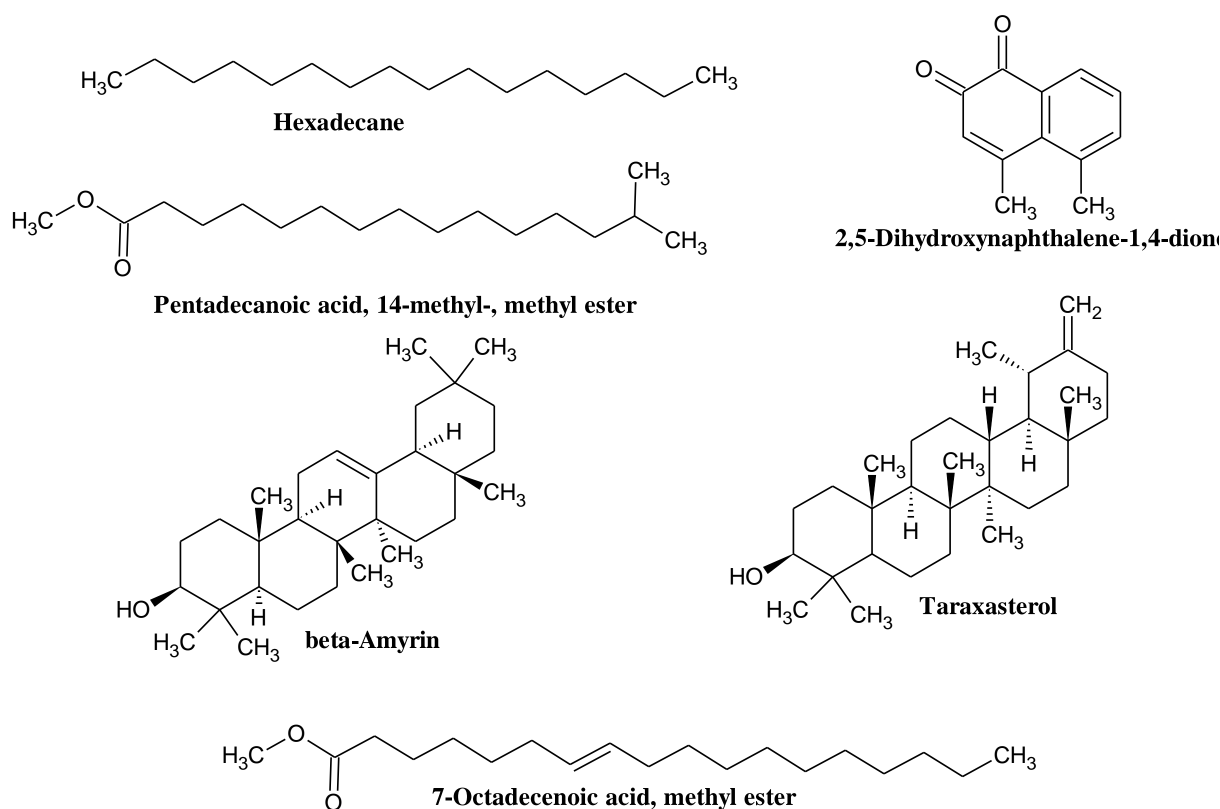

Figure 2. Chemical structure of the main compounds identified by the GC-MS analyses in the chloroform extract of P. Crispa.

There were 35 detected compounds, which constitute 77.54 % of the whole extract. The most dominant of all the identified compounds were 2,5-Dihydroxynaphthalene-1,4-dione (16.19%), Pentadecanoic acid, 14-methyl-, methyl ester (12.37%), Hexadecane (8.99%), while the most representative compounds identified were 7-Octadecenoic acid, methyl ester (5.13%), Octadecanoic acid, methyl ester (4.69%), Taraxasterol (4.19%), n-Hexadecanoic acid (3.11%), beta-Amyrin (3.26%), 4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)-tyramine (2.39%) and Diisooctyl phthalate (2.46%).

The other constituents present in appreciable amounts were β-caryophyllene (0.56%), acetol (0.64%), linalyl formate (1.93%) and α-tocopherol-β-D-mannoside (1.78%).

The presence of newly identified compounds especially 2,5-Dihydroxynaphthalene-1,4-dione (16.19%) or their combination with other secondary metabolites might contribute to the specific bioactivity related to antioxidant, anti-glycation, anti-acetylcholinesterase and other processes.

Compound 27 ($C_{10}H_6O_4$) 2,5-Dihydroxynaphthalene-1,4-dione, which is also known as Isonaphthazarin, has the highest percentage (16.19%), followed by the two compounds: compound 19; Pentadecanoic acid, 14-methyl-, methyl ester, and compound 14 Hexadecane ($C_{16}H_{34}$) that have 12.37% and 8.99%, respectively. The 7-Octadecanoic acid, methyl ester (compound 24), was

extracted from plants like *Cymbopogon nardus* and *Plumbago zeylanica,* and showed promising medicinal uses. A scientific report indicated that octadecanoic acid, methyl ester exhibited antiviral properties, as showing its effectiveness against measles virus when combined with ribavirin [23].

Hexadecanoic acid (compound 20) ($C_{16}H_{32}O$), present in the extract at a rate of 3.11%, also known as palmitic acid, has exhibited various medicinal properties as highlighted previous research papers. These studies have shown that hexadecanoic acid isolated from marine hydroids can effectively inhibit the growth of fungi responsible for fruit spoilage, such as *Botrytis cinerea* and *Rhizopus stolonifer* on strawberries, and *Aspergillus niger* on mangoes [24]. Furthermore, hexadecanoic acid has demonstrated antibacterial activity against *Salmonella typhi*, making it a potential candidate for combating infectious diseases caused by pathogenic microorganisms [25]. These findings suggest that hexadecanoic acid and its derivatives hold significant potential for medicinal applications in antifungal, antibacterial, and anticancer therapies.

Podocarp-13-en-12-ol ($C_{17}H_{28}O$), a compound related to podocarpic acid, showed promise for various medicinal applications based on the research findings. Studies have highlighted the biological activities of compounds similar to podocarpic acid, including anti-leukemic properties, inhibition of plant cell growth, insect toxicity, and antifungal effects [26] . Additionally, *Podocarpus nagi* extracts containing norditerpenoid compounds have demonstrated significant activity in resisting pulmonary fibrosis, suggesting a potential role in respiratory health treatments [27]. Furthermore, research on *Podocarpus nagi* has explored its anticancer potential, indicating that compounds from this plant could serve as effective anticancer agents with fewer side effects compared to synthetic drugs [28]. Therefore, Podocarp-13-en-12-ol holds promise for medicinal use in areas such as cancer treatment, respiratory health, and liver cancer prevention.

Beta-amyrin ($C_{30}H_{50}O$), a natural compound found in various plant extracts like *achyranthes aspera*, *calotropis gigantea*, and *celastrus hindsii*, exhibited a wide range of medicinal properties. This compound has exhibited an important potential in treating tuberculosis by targeting mycobacterium proteins, particularly interacting strongly with Rv1636 to make *mycobacterium tuberculosis* vulnerable to host-derived stress conditions [29]. Additionally, beta-amyrin has shown interesting effect in preventing gout and skin hyperpigmentation, making it a potential candidate for future therapies [30]. Furthermore, beta-amyrin has demonstrated neuroprotective effects against Alzheimer's disease by ameliorating memory impairment, improving signaling pathways, and enhancing object recognition memory in AD mouse models [31]. Moreover, beta-amyrin has been found to exert a nephroprotective effect against glycerol-induced nephrotoxicity in rats, showcasing its potential in protecting against acute kidney injury [32]. Lastly, beta-amyrin exhibited significant anticancer activity against liver cancer cells, inducing apoptosis, G2/M cycle arrest, and activating

specific signaling pathways, suggesting its potential as a lead molecule for liver cancer treatment [33]. Taraxasterol ($C_{30}H_{50}O$), a triterpenes found in many plants of the Asteraceae family, exhibited promising medicinal properties across various health conditions. Researchers indicated that taraxasterol showed a potential in inhibiting prostate cancer cell metastasis by reducing cell survival rates, gene expressions related to cancer progression, and enzyme activity levels [34]. Moreover, taraxasterol demonstrated the ability to alleviate cardiac aging, fibrosis, and insulin signaling desensitization, suggesting its effectiveness in treating aging-related cardiac diseases[35]. In dairy cows, taraxasterol has shown anti-inflammatory and antioxidant effects, potentially alleviating fatty liver by reducing lipid accumulation, oxidative stress, and endoplasmic reticulum stress in hepatocytes [36]. Besides, taraxasterol exhibited anti-tumor properties by inducing cell cycle arrest, preventing cell migration, and promoting cancer cell apoptosis, making it a potential natural drug for lung cancer therapy [37]. These findings collectively highlight the diverse medicinal uses of taraxasterol in combating various health issues.

12-Oleanen-3-yl acetate, (3.alpha) ($C_{32}H_{28}O$), ($C_{32}H_{52}O_2$) a derivative of oleanolic acid, shows promising medicinal potential. Research indicates that oleanolic acid, the parent compound, possesses hepatoprotective, antitumor, antiviral, anti-inflammatory, and antioxidant properties [38], [39]. Furthermore, a study on a novel derivative of oleanolic acid, N-[(3beta)-3-(acetyloxy)-28-oxoolean-12-en-28-yl]-glycine methyl ester, demonstrated potent anti-tumor activity against melanoma and leukemia cells, inducing cell cycle arrest and inhibiting cell growth [40]. Additionally, oleanolic acid itself has been identified as a vasodilator and restorer agent for endothelial dysfunction, showcasing its potential therapeutic application in cardiovascular health [41]. These findings collectively suggest that 12-Oleanen-3-yl acetate, (3.alpha), and its derivatives possess various medicinal uses, including anti-inflammatory, anti-tumor, hepatoprotective, and cardiovascular applications.

Anti-AGEs

The results of Anti-AGEs activities (IC50) of the CHCl3, EtOAc and BuOH extracts obtained from aerial parts of P. crispa and the reference compound amino-guanidine were summarized in Table 2.

Table 2: Anti-AGEs activities (IC50) of the CHCl3, EtOAc and BuOH extracts obtained from aerial parts of *P. crispa* and the reference compound aminoguanidine

	335/385 nm Pentosidine-like AGE inhibition formation	**370/440 nm AGEs inhibition formation**
	(IC_{50}: mg/ml)	

Aminoguanidine*	0.08	0.77
$CHCl_3$ extract	> 1	0.79
EtOAc extract	0.17	0.07
n-BuOH extract	0.15	0.41

*: reference compound

The authors assert that is the first study reporting P. cripa Anti-AGEs activity. The reactivity of plant extracts against AGEs is an intriguing and promising area of scientific research, particularly in the context of addressing complications associated with diabetes and aging [14]. AGEs are formed through a non-enzymatic reaction between reducing sugars and proteins, lipids, or nucleic acids. They play a crucial role in the pathogenesis of various chronic diseases, including diabetes mellitus, neurodegenerative disorders, and cardiovascular diseases. Several studies have investigated the potential of plant extracts to inhibit the formation of AGEs or mitigate their adverse effects. The bioactive compounds present in plants, such as polyphenols, flavonoids, and other antioxidants, have been identified as key players in this regard [42], [43].

Many plant extracts are rich in antioxidants that can neutralize reactive oxygen species (ROS) and prevent oxidative stress, a process linked to AGE formation. Polyphenols, found abundantly in green tea, fruits, and vegetables. It has been reported that they possess strong antioxidant properties and can inhibit the formation of AGEs [44]. Some plant extracts exhibit direct anti-glycation activity by interfering with the Maillard reaction, which leads to the formation of AGEs. Certain compounds, like aminoguanidine and thiamine derivatives found in some plants, have been approved for their ability to inhibit glycation reactions [45]. Plant extracts may contain compounds that inhibit the activity of enzymes involved in the glycation process, such as aldose reductase. For example, extracts from bitter melon have shown inhibitory effects on aldose reductase, potentially reducing the formation of AGEs [46]. Chronic inflammation is closely linked to the formation of AGEs. Plant extracts with anti-inflammatory properties may indirectly mitigate AGE formation. Curcumin, derived from turmeric, has demonstrated both anti-inflammatory and anti-glycation effects [47]. AGEs contribute to tissue damage by cross-linking proteins. Plant extracts may interfere with this process, preventing the development of complications. Compounds like resveratrol, found in red grapes and berries, demonstrated their potential to inhibit protein cross-linking [48]. Plant extracts may protect cells from the damaging effects of AGEs, preventing or alleviating complications associated with their accumulation. The use of plant extracts in cell culture models has demonstrated protective effects against AGE-induced cellular damage [49].

It is important to note that the effectiveness of plant extracts can vary depending on the source, extraction methods, and the specific bioactive compounds present. While there is promising evidence supporting the use of plant extracts in combating AGE-related complications, further research, including clinical trials, is needed to establish their therapeutic potential and optimal utilization. Additionally, interdisciplinary collaboration between pharmacologists, biochemists, and clinicians is essential to translate these findings into practical therapeutic strategies. P. crispa EtOAc and BuOH extracts showed an interesting ability to inhibit the formation of pentosidine and IC50 = 0.17 and 0.15 (mg/mL), respectively) which were nearer to the standard potential (IC50 = 0.08 (mg/mL)). Nevertheless, the potential of the same extracts (EtOAc and BuOH) was better (IC50 = 0.07 mg/mL and 0.41 mg/mL, respectively) than aminoguanidine effect in the inhibition of other AGEs products formation (IC50 = 0.77). The CHCl3 extract also showed an important ability to stop the formation of pentosidine and other AGEs products formation (IC50 > 1 and 0.79 (mg/mL), respectively). So, our plant extracts had a high ability to stop the formation of advanced glycation end products, thus avoiding many chronic diseases such as those mentioned above.

Anti-acetylcholinesterase

Acetylcholinesterase inhibitors temporarily improve the symptoms Alzheimer disease by stopping acetylcholine from breaking down in the body. These inhibitors block the action of the enzyme acetylcholinesterase, which is responsible for breaking down acetylcholine. This raises the concentration of acetylcholine in the area between nerve endings and provides it more chances to transmit the nervous impulse. This mechanism is important for people with Alzheimer's disease, who already have low levels of acetylcholine, because these inhibitors temporarily improve the symptoms of the disease and its stability [50]. The evaluation of the inhibitory effect of the acetylcholinesterase enzyme resulted with an IC50 equal to 7.20±1.01 and 7.78±0.72 µg/ml that correspond to n-butanol and ethyl acetate extracts activities, respectively. These outcomes are very close to positive control (IC50 = 6.27 ± 1.15 µg/ml) Table 3.

Table 3: IC50 values of anti-acetylcholinesterase and antioxidant activities.

Extract/standards	DPPH ($IC_{50 \mu g}$)	Metal Chelating ($IC_{50 \mu g}$)	Superoxide anion ($IC_{50 \mu g}$)	Reducing power ($A_{0.5 \mu g}$)	AchE
$CHCl_3$	>50	106.35±8.02 [c]	33.94±7.6 [a]	> 50	60.17±1.03 [a]
EtOAc	3,10±0.12 [d]	602.34±25.36 [a]	<3.125	29,69±3.37 [b]	7.78±0.72 [b]

n-BuOH	6,82±0.22[b]	232.5±12.25[b]	1.22±1.45[b]	38,92±3.82[a]	7.20±1.01[c]
BHA	6.14 ± 0.41[c]	-	-	-	-
BHT	12.99± 0.41[a]	-	-	-	-
Ascorbic acid	-	-	-	6.77 ± 1.15[c]	-
EDTA	-	8.80 ± 0.47[d]	-	-	-
Galantamin	-	-	-	-	6.27 ± 1.15[d]

-: not tested, IC50 and A 0.5 are expressed as mean ± SD (n = 3). The values with different superscripts (a , b , c , d , e) in the same column are significantly different ($p < 0.05$).

The strong effect of P. crispa extracts on AChE activity can be due to the chemical arsenal of this species, since we believed that the main substance in a plant extract is generally responsible for all of its bioactivities, notably beta-amyrin [31]. The present work represent the first study, because, still now, no scientific report dealed with the potential of P. crispa metabolites to inhibit the acetylcholinesterase enzyme. Nevertheless, essentials oils and volatile compounds from others species such as, *Pulicaria undulata* and *Pulicaria stephanocarpa* have been found to possess inhibitory effects regarding acetylcholinesterase enzyme and researchers claim that is owing to the effect of their major compound carvotanacetone and (E)-Caryophyllene [51], [52].

Antioxidants activities

Fruits and vegetables provide extremely powerful levels of antioxidants, are readily available, are not prohibitively expensive, are much more dependable than any synthetic medication now on the industry, do not cause any adverse side effects, and possess an excellent safety rating [53]. The relevance of antioxidants derived from plants has been demonstrated by a significant number of research investigations[54]'[55]'[56]'[57]'[58]'[59]'[60]. In order to obtain data that were more instructive regarding the antioxidant activity of P. crispa extracts, it was required to examine them using a number of different experiments. As consequence, the antioxidant activity was evaluated using a total of four different tests: the DPPH and ABTS free radical scavenging assays, the reducing power, and the metal chelating activities. According to the findings presented in Table 3, the ethyl-acetate extract was significantly more powerful compared to the other extracts regarding DPPH and reducing power assays. In fact, the ethyl-acetate extract performed even better than the standard BHA in the DPPH assay. The n-butanol extract proved as the most effective antioxidant against the superoxide anion. In addition, we found that of P. crispa extracts exhibited a moderate antioxidant activity against metal chelation.

As aromatic plants, the essential oils of Pulicaria species have been extensively analyzed, revealing over 300 volatile organic compounds. Among these, oxygenated monoterpenes and sesquiterpenes are predominant. Similarly, flavonoids and sesquiterpenes are the most frequently reported compounds in the non-essential oil fractions. Various biological and pharmacological activities have been documented for the essential oils, crude extracts, and their fractions or isolated compounds of Pulicaria species. Among these, antimicrobial, anticancer, and antioxidant activities have been predominantly investigated, primarily under in vitro conditions. Numerous distinctive compounds have been identified for each species, which can potentially serve as chemical markers in their characterization [61]. An earlier study aimed to investigate the active phytochemicals present in the methanolic extract of P. crispa. Significant antimicrobial and antioxidant activities were observed, which may be attributed to the presence of tannins, phenols, and flavonoids in the methanolic extract. The antioxidant potential of this plant from Saudi Arabia was less efficient that the one of all P. crispa extracts test of our Algerian plant [62]. This the first investigation that performed many antioxidant assays and for different extraction fractions. Indeed, the majority of studies [63], [64], [65] were carried on P. crispa essential oils and the outcomes demonstrated that the IC50 values were elevated than those of the extracts tested in the current work, which claimed that the plant of interest from Algerian Sahara is more potent in term of antioxidant activity.

Conclusion

The GC-MS analysis identified approximately 35 compounds from the aerial parts of the chloroform extract of P. crispa. Notably, the n-butanol, ethyl acetate, and chloroform extracts demonstrated potent antioxidant, acetylcholinesterase inhibitory, and anti-AGEs activities. These effects can be attributed to their high concentrations of bioactive substances, such as polyphenols, flavonoids, triterpenoids, alkaloids, and others, which have been previously reported in this plant. Our findings, when compared to studies of the same plant from other regions, suggest that the Algerian species from a hot and arid desert climate exhibits superior antioxidant potential.

The novelty of our research lies in the first-time evaluation of the anti-acetylcholinesterase and anti-AGEs activities of P. crispa. Remarkably, the extracts displayed exceptional inhibitory effects on acetylcholinesterase and AGEs formation, with some extracts matching or even surpassing the performance of reference compounds. However, further studies are essential to isolate specific active molecules, especially the major ones, and to assess their individual activities. Additionally, detailed *in vivo* and clinical trials are necessary to substantiate their potential as therapeutic agents.

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Competing interests

The authors declare that they have no competing financial interests or conflict of interest.

Ethical Approval

Not applicable.

Informed Consent

Not applicable.

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