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Research article

Phytochemical characterization and multifaceted bioactivity assessment of essential oil from *Ptychotis verticillata* Duby: Anti-diabetic, anti-tyrosinase, and anti-inflammatory activity

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ABSTRACT

The aim of this study is to explore the pharmacological properties of the essential oil derived from *Ptychotis verticillata* Duby (PVEO), a medicinal plant native to Morocco, focusing on its antidiabetic, anti-tyrosinase, and anti-inflammatory effects. Additionally, the study aims to characterize the phytochemical composition of PVEO and evaluate its potential as a natural therapeutic alternative for various health conditions. To achieve this, phytochemical analysis was conducted using gas chromatography-mass spectrometry (GC-MS). Furthermore, in vitro assessments were conducted to investigate PVEO's antidiabetic activity by inhibiting α-amylase, xanthine oxidase, and α-glucosidase. Tests were also undertaken to evaluate the anti-inflammatory effect of PVEO on RAW 264.7 cells stimulated by lipopolysaccharide (LPS), as well as its efficacy as an anti-tyrosinase agent and its lipoxygenase inhibition activity. The results of the phytochemical

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analysis revealed that PVEO is rich in terpene compounds, with percentages of 40.35 % γ-terpinene, 22.40 % carvacrol, and 19.77 % β-cymene. Moreover, in vitro evaluations demonstrated that PVEO exhibits significant inhibitory activity against α-amylase, xanthine oxidase, and α-glucosidase, indicating promising antidiabetic, and anti-gout potential. Furthermore, PVEO showed significant anti-tyrosinase activity, with an IC50 of 27.39 ± 0.44 μg/mL, and remarkable lipoxygenase inhibition (87.33 ± 2.6 %), suggesting its candidacy for dermatoprotection. Additionally, PVEO displayed a dose-dependent capacity to attenuate the production of NO and PGE_2, two inflammatory mediators implicated in various pathologies, without compromising cellular viability. The findings of this study provide a solid foundation for future research on natural therapies and the development of new drugs, highlighting the therapeutic potential of PVEO in the treatment of gout, diabetes, pigmentation disorders, and inflammation.

1. Introduction

For decades, essential oils (EOs) sourced from many plants have been extensively employed in conventional medicine [1]. They are rich in bioactive compounds, such as terpenes, phenols, and sesquiterpenes, that exhibit diverse biological and pharmacological activities [2]. Among these activities, anti-diabetic, anti-tyrosinase, and anti-inflammatory effects are of particular interest, as they may offer natural alternatives for the management of prevalent and challenging health conditions [3,4].

Millions of individuals throughout the world are afflicted with diabetes, a chronic metabolic illness [5]. Consisting of chronic hyperglycemia and a range of consequences, it is distinguished by malfunction in glucose metabolism [6]. Diabetes can be classified into two main types: Type 1, which is of genetic and autoimmune origin and requires insulin therapy [7], and Type 2, which is often associated with lifestyle factors such as obesity and physical inactivity, and can be controlled by dietary changes, exercise, and oral medications [8,9]. Several studies have suggested that some EOs may have blood sugar regulatory properties, possibly by modulating insulin secretion or sensitivity [10,11].

Tyrosinase is an enzyme implicated in the melanin synthesis, the pigment responsible for skin, hair, and eye color [12,13]. Melanin plays a vital role in protecting the skin from ultraviolet radiation and oxidative stress [14]. However, abnormal tyrosinase activity or expression can lead to pigmentation disorders, such as albinism, vitiligo, melasma, and hyperpigmentation [15]. These disorders can cause cosmetic and psychological problems [16,17]. Hence, tyrosinase activity inhibition is a prevalent approach utilized in the management and prophylaxis of pigmentation disorders [18]. Certain EOs have been shown to have anti-tyrosinase action; this might be due to the scavenging free radicals or interfering with the enzyme's catalytic mechanism [19–21].

Inflammation is a complex and dynamic process that occurs in response to tissue injury or infection, it is essential for the elimination of pathogens and the repair of damaged tissues [22]. However, when inflammation becomes chronic or excessive, it can cause or aggravate various diseases, such as arthritis, asthma, and atherosclerosis [23]. Inflammatory pathways are frequently stimulated in the context of chronic inflammation and the production of pro-inflammatory mediators, such as prostaglandins, chemokines, and cytokines [24]. Several EOs have been shown to possess anti-inflammatory properties, possibly by modulating the expression or activity of these mediators or by affecting the signaling pathways that regulate inflammation [25–28].

The essential oil extracted from *Ptychotis verticillata*, also known as *Ammoides verticillata* (Brot.) Breistr, a medicinal and aromatic plant endemic to Morocco and belonging to the Apiaceae family, has attracted considerable attention due to its therapeutic potential [29]. The Apiaceae family is recognized for its richness in bioactive compounds, with their essential oils containing phenylpropanoids such as thymol, carvacrol, estragole, and anethole, which possess antioxidant properties [30]. These molecules have sparked significant interest in medical research owing to their bioactivity, including their ability to neutralize free radicals and protect cells against oxidative damage [31]. Traditionally referred to as "Nûnkha," this plant is utilized for various purposes such as flavouring [32], perfumery, and the treatment of digestive and respiratory disorders. Several studies have highlighted the abundance of terpene compounds in this plants essential oil, including carvacrol, thymol, D-limonene and gamma-terpinene [29,33,34]. Other studies have demonstrated the antioxidant, antibacterial, and anticancer efficacy of this essential oil, particularly in the treatment of breast cancer [29,35]. Another study on the synergy of this essential oil with conventional antibiotics such as ampicillin, amoxicillin, and erythromycin showed promising results. These combinations reduced the minimum inhibitory concentration of these antibiotics up to 10 folds against a wide range of bacteria [34]. However, the anti-diabetic, anti-tyrosinase, and anti-inflammatory activities of PVEO remain insufficiently explored in the scientific literature.

Therefore, the objective of this study is to characterize the phytochemical profile of PVEO obtained from the eastern region of Morocco and to evaluate its anti-diabetic, anti-tyrosinase, and anti-inflammatory effects. These comprehensive investigations, aim to contribute to the understanding of the therapeutic potential of this natural resource and to provide new insights for the development of novel and effective agents for diabetes management, pigmentation disorders, and chronic inflammation.

2. Materials and methods

2.1. Plant material and essential oil extraction

Harvested in 2022, the *Ptychotis verticillata* Duby, native to the Ahfir area in Eastern Morocco, was purchased at a local market in Oujda, Eastern Morocco. Botanical identification of the specimen was carried out by expert botanist Professor Mohamed Addi,

researcher at the Oujda Faculty of Science. A voucher number (CLP-003) was assigned to the specimen at the Center for Oriental Water and Environmental Science and Technology of Mohammed 1st University of Oujda. Subsequently, the essential oil was systematically extracted from the aerial components of the plant by the utilization of hydrodistillation with a modified Clevenger equipment. This selected methodology rigorously adheres to the established protocols [29,36].

2.2. Phytochemical composition by GC-MS

Using a gas chromatograph coupled with a mass spectrometer detector, qualitative and semi-quantitative analysis of the essential oil derived from *Ptychotis verticillata* Duby was conducted, as described by El Guerrouj et al. [37]. The chemicals were identified and separated utilizing a Shimadzu GC system manufactured in Kyoto, Japan, in conjunction with an MS QP2010 equipment available from Fuji Scientific Instruments in Kyoto, Japan. The process of separation was carried out by employing a BPX5 capillary column that consisted of 5 % phenyl and 95 % dimethylpolysiloxane. The carrier gas utilized in this experiment was pure helium (99.99 percent). To ensure a consistent flow rate of 1.69 mL/min, the injection, interface temperatures, and ion source were all held constant at 250 °C. Programming the column heating system to increase at a rate of 10 °C per minute from 50 °C (for 1 min) to 250 °C (for 1 min), maintaining the temperature for 1 min. The components of the sample were ionized using Electron Impact (EI) mode at an energy of 70 eV. The mass range that was analyzed varied between 40 and 300 *m/z*. Following that, 1 μL of each produced oil was injected with a fractionation ratio of 90:1. Each sample underwent three assessments, during which chemical identification was achieved by comparing retention durations to validated standards and mass spectrum fragmentation models derived from databases or NIST compounds [38]. The method of collecting and processing data was carried out using Laboratory Solutions (v2.5).

2.3. Antidiabetic activity

2.3.1. α-Amylase inhibition assay

In the examination of potential antidiabetic properties inherent in *Ptychotis verticillata* essential oil (PVEO), the α-amylase inhibition assay was meticulously conducted. Compounds isolated from PVEO underwent a pre-incubation with α-amylase solution (1 U/mL) for 10 min at 37 °C. The reaction was begun by introducing 30 μL of soluble starch (0.5 % concentration in deionized water) and thereafter incubating it at 37 °C for 6 min. The reaction was carefully terminated, and absorbance at 565 nm was precisely measure [39–41].

2.3.2. α-Glucosidase inhibition assay

Simultaneously, the α-glucosidase inhibition assay was rigorously executed. A methanolic stock solution, combined with 0.5 U/mL of α-glucosidase enzyme solution and potassium phosphate buffer (0.1 M), underwent a 6-min incubation at 25 °C. Next, 20 μL of a solution of *p*-nitrophenyl-α-D-glucopyranoside substrate (5 mM) was added, and the mixture was incubated for 8 min. The process was carefully halted, and the absorbance was accurately measured at a wavelength of 405 nm [41].

2.3.3. Xanthine oxidase (XO) assay

Introducing an additional dimension, the xanthine oxidase (XO) assay explored the inhibitory potential of PVEO on xanthine oxidase. Conducted under aerobic conditions, the assay mixture underwent a 15-min preincubation at 25 °C. The reaction was started by introducing the substrate solution, consisting of 150 mM xanthine in a 70 mM phosphate buffer, and allowing it to incubate at a temperature of 25 °C for a duration of 30 min. The process was deliberately stopped, and the absorbance at 290 nm was accurately determined [42].

2.4. Dermatoprotective activity: tyrosinase inhibition assay

In the investigation of the dermatoprotective potential inherent in *Ptychotis verticillata* essential oil (PVEO), a methodological adaptation was employed to assess tyrosinase inhibitory activity [43,44]. In a succinct delineation, 25 μL of the PVEO sample was meticulously amalgamated with 100 μL of tyrosinase solution (333 U/mL, 50 mM phosphate buffer, pH 6.5), undergoing incubation at 37 °C for a precise interval of 10 min. After this initial phase, 300 μL of L-DOPA (5 mM) was introduced, and the resulting mixture underwent a subsequent 30-min incubation at 37 °C. The resultant absorbance readings were meticulously recorded at 510 nm utilizing a UV-VIS spectrophotometer (Jenway 6300, Staffordshire, UK). Tyrosinase inhibition levels were quantified across a spectrum of PVEO concentrations, specifically at 10, 20, 40, 60, 120, and 160 μg/mL. The determination of IC50 values, representing the concentration at which 50 % inhibition occurred, formed a pivotal aspect of the analytical framework. It is imperative to note that quercetin served as the positive control in this experimental context, providing a benchmark for the assessment of tyrosinase inhibitory activity. This meticulous methodological approach facilitated a comprehensive evaluation of PVEO's capacity to inhibit tyrosinase, thereby contributing nuanced insights into its dermatoprotective attributes.

2.5. Anti-inflammatory activity of PVEO

2.5.1. Inhibition of 5-LOX enzyme

With a few minor modifications, this assessment adhered to the protocol described by Pinto et al. (2007) [45]. A UV-VIS spectrophotometer (Jenway 6300, Staffordshire, UK) with a 560 nm setting was used to measure the formation of the Fe^{3+}/xylenol orange

combination, which served as the basis for the assessments. For 5 min, the Glycine max enzyme 15-lipoxygenase was incubated at 25 °C with either plant extracts or a conventional inhibitor. After that, linoleic acid was added to 50 mM Tris–HCl buffer (pH 7.4) at a final concentration of 140 μM, and the mixture was incubated at 25 °C for 20 min in the dark. To stop the process, add 100 μL of FOX reagent, which is 30 mM sulfuric acid, 100 μM xylenol orange, and 100 μM iron (II) sulfate in a 9:1, v/v methanol/water solution.

Only the buffer and LOX solution were pipetted in the control wells. During the incubation period, the LOX enzyme was present in the blanks, and the substrate linoleic acid was added after the FOX reagent. After 30 min at 25 °C, changes in absorbance values at 560 nm were used to calculate the % suppression of hydrogen peroxide formation, which served as an indicator of lipoxygenase's inhibitory action. The following formula was used to calculate the inhibition percentage:

$$\text{Inhibition percentage } (\%) = \left[\frac{(A_{control} - A_{blank}) - (A_{sample} - A_{blank})}{(A_{control} - A_{blank})}\right]$$

The variable $A_{control}$ represents the absorbance value of the control well, A_{blank} represents the absorbance value of the blank well, and A_{sample} represents the absorbance value of a sample well.

2.5.2. Modulation of the inflammation in LPS-treated RAW 264.7 cells, and inhibition of major inflammation mediators

a Cell Culture

The investigation included culturing RAW 264.7 murine macrophage cells obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) in accordance with ATCC standards. Maintained at 37 °C in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), 4.5 g/L glucose, 4.5 mM glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin, the cells formed the basis for a thorough investigation.

b Cell Viability Assessment

Cell viability was meticulously evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay. Initially, RAW 264.7 cells were seeded at 1×10^4 cells per well in 96-well plates [46,47]. Subsequently, varying concentrations of PVEO were applied, ranging from 25 to 200 μg/mL, with or without lipopolysaccharide (LPS) at 1 μg/mL for 24 h. Post-treatment, MTT solution (10 μg/mL) was introduced, and formazan crystals were solubilized in dimethyl sulfoxide (DMSO). Absorbance readings at 570 nm, with a background control at 630 nm, were recorded using an ELISA microplate reader (BioTek Instruments, Inc, Winooski, VT, USA). The viability of the cells was assessed by determining the proportion in relation to the untreated cells.

c. Inflammatory Mediator Determination

Table 1
Phytochemical constituents of PVEO.

N°[a]	Compounds[b]	RT (min)	Area (%)	R_i^c	R_i Lit.[d]
1	α-Thujene	5.084	0.26	928	931
2	α -Pinene	5.214	2.79	933	939
3	β-Pinene	5.943	0.51	974	980
4	β-Myrcene	6.116	1.25	987	991
5	α-Phellandrene	6.391	0.45	993	1005
6	2-Carene	6.591	1.34	1010	1011
7	β-cymene	6.740	19.77	1022	1025
8	β-thujone	6.920	1.18	1029	1029
9	γ-terpinene	7.300	40.35	1048	1062
10	isothujol	7.794	0.21	1153	1170
11	4-terpineol	9.335	1.81	1169	1177
12	α-terpineol	9.566	0.46	1175	1189
13	carvone	10.809	0.34	1239	1243
14	thymol	11.063	6.29	1279	1290
15	carvacrol	11.165	22.40	1294	1298
16	cyclopent-1-ene-3,5-diol	11.343	0.60	1325	–
		Identified compounds		**Percentage**	
	Hydrocarbon monoterpenes	8		66.72	
	Oxygenated monoterpenes	7		32.69	
	Others	1		0.60	
	Total identified (%)	**16**		**100**	

a In order of elution.
b components identified by KI and MS.
c Kovats index calculated from alkanes series on the MS capillary column (C8–C24).
d Kovats index/Retention index from data libraries (NIST).

The assessment of nitric oxide (NO) levels employed the Griess reaction assay [48]. RAW 264.7 cells, seeded at 1×10^4 cells per well, underwent treatment with LPS, either alone or in conjunction with PVEO (25–100 μg/mL), for 24 h. Culture supernatants were subjected to Griess reagent, and the ensuing intracellular nitrate levels, a stable product of NO, were quantified at 540 nm using an ELISA microplate reader (BioTek Instruments, Inc, Winooski, VT, USA). Simultaneously, intracellular prostaglandin E2 (PGE_2) levels were quantified through an enzyme immunoassay (EIA) kit from R&D Systems, following the manufacturer's protocols. This meticulous approach provided comprehensive insights into the modulation of NO and PGE2 production in response to PVEO under LPS-induced inflammatory conditions, significantly contributing to the elucidation of PVEO's potential anti-inflammatory properties.

3. Results and discussion

3.1. Phytochemical composition

Table 1, and Fig. 1 displays the results for the 16 components found in the sample. The relative abundance or amount of each component in the sample is represented by the % area. According to the table, the component with the biggest percentage area is γ-terpinene (40.35 %). This means it is the most prevalent component in the sample. Carvacrol (22.40 %), is the second most abundant component in the sample, with the biggest percentage area. β-cymene (19.77 %), thymol (6.29 %), and α-pinene (2.79 %) are other prominent components with relatively high percentage area values. Components with low percentage area values, on the other hand, include α-thujene (0.26 %), α-phellandrene (0.45 %), α-terpineol (0.46 %), and (−)-carvone (0.34 %). These components are present in lesser amounts in the sample. Taibi et al. found that *P. verticillata* essential oil contains a range of aromatic and terpene components, with carvacrol being the predominant ingredient [35]. A further investigation carried out by Bnouham et al. revealed that the crucial oil derived from *P. verticillate,* there were a total of 10 components discovered. The main constituents were thymol, D-limonene, gamma-terpinene, and *m*-cymene, which accounted for 95.86 % of the total [32]. These findings contribute to our understanding of the chemical composition of PVEO and its potential applications in various fields, including pharmacology and aromatherapy.

Table 2 presents the outcomes of investigations into the inhibitory impacts of essential oil derived from *Ptychotis verticillata* (PVEO) on three key enzymes: xanthine oxidase (E.C. 1.17.3.2), α-amylase (E.C. 3.2.1.1), and α-glucosidase (E.C. 3.2.1.20). These enzymes are involved in the metabolism of purines and carbohydrates, and their inhibition can help reduce the symptoms of gout and diabetes, respectively [49–51]. According to the table, PVEO exhibited significant inhibitory activity against all three enzymes in terms of IC_{50}, which is the concentration of a substance that inhibits 50 % of the enzyme activity, and lower values indicate higher potency. PVEO was compared with two positive controls: allopurinol, a standard drug for gout that inhibits xanthine oxidase, and acarbose, a standard drug for diabetes that inhibits α-amylase and α-glucosidase. The findings indicate that PVEO had a comparable inhibitory impact on xanthine oxidase to allopurinol, with IC50 values of 25.33 ± 0.67 and 23.34 ± 0.09 μg/mL, respectively. Also, PVEO had a similar inhibitory effect on α-amylase, with IC_{50} values of 32.34 ± 0.78 μg/mL, while the essential oil had a relatively important inhibitory activity on α-glucosidase compared to the standard drug (acarbose, 65.41 ± 2.10 μg/mL) at 43.22 ± 3.03 μg/mL. Analysis of the chemical composition revealed the presence of various aromatic and terpene compounds, with major constituents such as γ-terpinene, β-cymene, carvacrol, and thymol. These compounds are recognized for their diverse biological properties, encompassing antioxidant, anti-inflammatory, and antidiabetic effects [52,53]. Notably, carvacrol and thymol, classified as phenolic monoterpenoids characterized by a hydroxyl group attached to a benzene ring [54,55], have demonstrated potent inhibitory effects on α-glucosidase, α-amylase, and xanthine oxidase in prior studies [56–59]. This investigation represents the first study to assess the anti-diabetic activity of this essential oil, making it difficult to compare with other previous studies. However, the results obtained in this study corroborate the findings of other research on essential oils rich in bioactive molecules. For example, the study conducted by Khedhri et al. (2023) on eucalyptus essential oils from Tunisia revealed that *E. leptophylla* and *E. brevifolia* exhibited remarkable inhibition of α-amylase, with an IC50 of 0.88 mg/mL [60]. Similarly, the study by Kochti et al. (2024) confirmed the efficacy of essential oils rich in terpene molecules, highlighting the antienzymatic efficacy of *Callitris glaucophylla* essential oil [61].

The cumulative findings suggest that PVEO holds potential as a natural alternative for gout treatment and may emerge as a

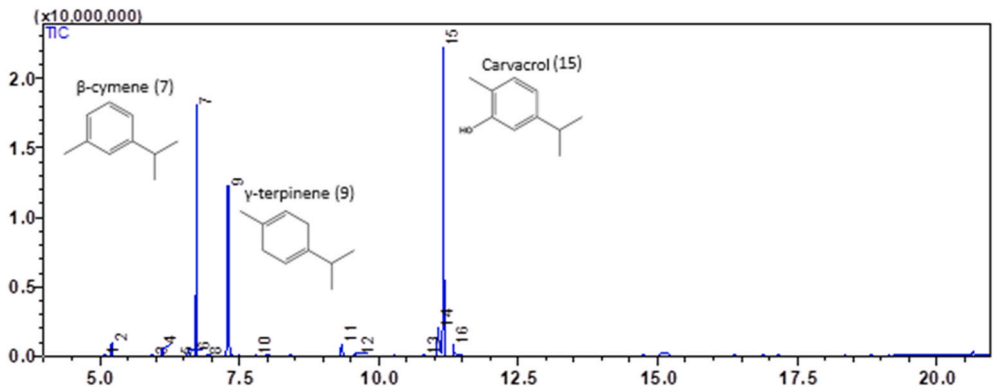

Fig. 1. GC-MS TIC chromatogram of *P. verticillata* essential oil (PVEO).

Table 2
α-Amylase, xanthine oxidase, and α-glucosidase inhibitory activities of (PVEO) in terms of IC_{50} values.

Essential oil/Positive control	IC_{50} (μg/mL), ±SD		
	Xanthine Oxidase Inhibition	α-Amylase Inhibitory Activity	α-Glucosidase Inhibitory Activity
PVEO	25.33 ± 0.67 a	32.34 ± 0.78 a	43.22 ± 3.03 b
Allopurinol[a]	23.34 ± 0.09 a	–	–
Acarbose[a]	–	35.48 ± 0.69 a	65.41 ± 2.10 a

[a] Positive controls. Values are reported as means ± SD (standard deviation); a, and b, designate significant difference in each column at $p < 0.05$.

promising natural agent for diabetes management.

3.2. Tyrosinase inhibition assay

The assessment of anti-tyrosinase activity assumes a pivotal role in the realm of dermatoprotection, given the central involvement of this enzyme in melanin biosynthesis. Melanin, the pigment dictating skin, hair, and eye coloration, arises from the conversion of tyrosine to DOPA catalyzed by tyrosinase [62]. This intricate process, recognized as melanogenesis, stands as a cornerstone for melanosome formation and melanin production, thereby fundamentally shaping skin pigmentation and texture [63].

In this investigation, the anti-tyrosinase activity of PVEO was meticulously examined through a tyrosinase inhibition assay (Table 3). The discerned results elucidate an anti-tyrosinase activity value of 27.39 ± 0.44 μg/mL for PVEO, juxtaposed against the control, quercetin, having a value of 22.15 ± 0.12 μg/mL. These findings underscore the nuanced capacity of PVEO to modulate tyrosinase activity, suggesting its potential in dermatoprotection, and the regulation of melanin synthesis. The noteworthy observation of anti-tyrosinase activity within PVEO may be ascribed to its rich terpenoid composition, exemplified by one of its major compounds, carvacrol, known for its demonstrated efficacy in inhibiting tyrosinase [64,65]. It is imperative to acknowledge the intricacies involved in comparing these results with extant studies, given the uniqueness of this research as the inaugural exploration specifically addressing the anti-tyrosinase activity of this plant. These outcomes not only lay the foundation for a more profound understanding of underlying mechanisms but also offer auspicious avenues for the application of PVEO in dermatology, promising advancements in products targeting hyperpigmentation challenges, and advocating for a balanced, and aesthetically beneficial skin pigmentation.

3.3. Anti-inflammatory activity results

3.3.1. LOX inhibition

The data from Table 4 highlight the remarkable lipoxygenase (LOX) inhibition capacity of PVEO, reaching 87.62 ± 2.60 % at a concentration of 0.5 mg/mL. In parallel, quercetin, a well-known chemical compound with inhibitory properties, exhibits a LOX inhibition capacity evaluated at 93.59 ± 1.19 % at a concentration of 1 mg/mL. It is essential to note that the LOX inhibition capacity of the essential oil (0.5 mg/mL) surpasses that observed with quercetin (1 mg/mL), suggesting that the essential oil of *P. verticillata* could be considered a potentially more effective inhibitor of LOX, even more potent than quercetin under the experimental conditions.

The anti-inflammatory activity of the essential oil of the plant can be largely attributed to its composition of bioactive molecules. γ-terpinene, one of the major bioactive compounds in this essential oil, has been identified as an inhibitor of 5-LOX [66], demonstrating its ability to modulate acute inflammation in various *in vivo* experimental models [67]. Furthermore, studies suggest that carvacrol, the principal compound in this essential oil, may also possess anti-inflammatory properties by inhibiting certain inflammation mechanisms [68–71]. The results emphasize the encouraging capacity of PVEO as a natural anti-inflammatory substance.

3.3.2. Anti-inflammatory activity on LPS-induced NO, and PGE2 production in RAW 264.7 cells

Assessing the survival of RAW 264.7 cells triggered with lipopolysaccharide (LPS) is an important step in understanding the impact of PVEO on the inflammatory response. As depicted in Fig. 2, the RAW 264.7 cell percentage showed a notable rise following exposure to 1 μg/mL of LPS. Conversely, the quantity of RAW 264.7 cells remained unaffected by escalating concentrations of PVEO, except for the fourth concentration (150 μg/mL), which exhibited a minor decrease in cell viability to 96.80 ± 2.04 %. Notably, doses up to 200 μg/mL did not elicit any reduction in cell viability, suggesting the non-cytotoxic nature of PVEO towards RAW 264.7 cells.

This study seeks to appraise the anti-inflammatory potential of PVEO by influencing the production of inflammatory mediators, notably nitric oxide (NO) and prostaglandin E2 (PGE2). It is crucial to highlight the significance of NO and PGE2 in the anti-inflammatory process, where their inhibition assumes a pivotal role in regulating inflammatory responses [72,73]. NO, an essential regulator of inflammation-related cellular cascades [74], and PGE_2, closely associated with mediating inflammatory responses and

Table 3
In vitro dermatoprotective activity using Tyrosinase inhibition assay.

Assay	PVEO	Control (Quercetin)
	IC_{50} (μg/mL)s	
Tyrosinase	27.39 ± 0.44	22.15 ± 0.12

Table 4
Results of LOX inhibition (%) of the *P. verticillata* essential oil.

Sample (0.5 mg/mL)	% LOX inhibition
PVEO	87.62 ± 2.60
Quercetin (1 mg/mL)	93.59 ± 1.19

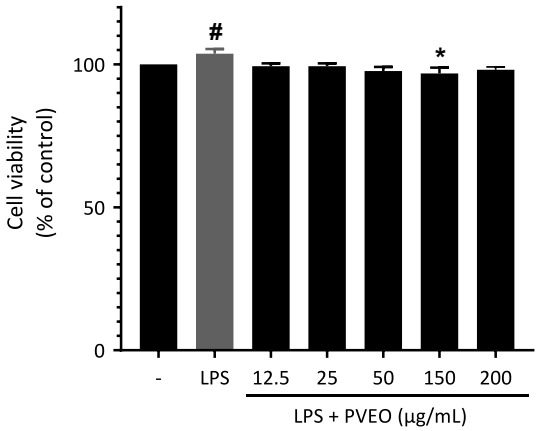

Fig. 2. Effects of PVEO on the viability of RAW 264.7 cells. The cells were treated with LPS at a dose of 1 μg/mL and incubated for 24 h in the presence or absence of increasing concentrations (25–200 μg/mL) of PVEO. The assessment of cell viability was conducted using the MTT colorimetric test. # denotes a significant difference between the control, and LPS-only treated groups at $p < 0.05$. * denotes a significant difference between the control, and LPS-PVEO treated groups at $p < 0.05$.

sensitizing nociceptors, emphasizes the importance of targeting these mediators [75]. The results in Fig. 3A and B expound upon the concentrations measured under specific experimental conditions. Fig. 3A delineates that the control group manifests a basal NO production of 4.40 ± 0.43 μM while introducing lipopolysaccharide (LPS) induces a substantial increase to 45.76 ± 1.88 μM. The simultaneous addition of PVEO, spanning concentrations from 25 to 200 μg/mL with LPS at a dose of 1 μg/mL, demonstrates a dose-dependent reduction in NO production, ranging between 39.07 ± 0.90 and 15.77 ± 2.47 μM. Analogously, in Fig. 3B, the control group exhibits a PGE_2 production of 54.20 ± 2.77 pg/mL, while LPS alone significantly amplifies this production to 718.50 ± 7.26 pg/mL. The introduction of PVEO at varying concentrations induces a dose-dependent decline in PGE_2 production, ranging from 513.50 ± 8.97 to 111.60 ± 4.63 pg/mL.

These outcomes posit that PVEO harbors anti-inflammatory activity, exemplifying a dose-dependent efficacy in attenuating LPS-induced NO and PGE_2 production. These findings underscore the advantageous potential of PVEO in modulating inflammatory processes. The assays reveal a dose-dependent decrease in NO and PGE_2 production in the presence of PVEO, thereby illustrating its capacity to modulate inflammatory responses. This noteworthy activity is likely attributed to bioactive compounds, with carvacrol emerging as one of the predominant constituents in this essential oil [75,76]. This capacity to mitigate these inflammatory mediators suggests a promising anti-inflammatory effect of PVEO.

All these biological activities can be attributed to their richness in volatile compounds. These volatile chemicals play an essential role in pharmacology, particularly in natural products, essential oils (EO) and fragrance compounds [77]. They are often endowed with biological actions that can have an impact on human health. Synergistic effects are frequently observed when several volatile components of plant extracts are combined [78]. Compared to individual drugs, the interaction between these substances can increase their total pharmacological activity. Thymol, carvacrol and β-cymene are examples of volatile chemicals with anti-inflammatory activities. These chemicals have the ability to modulate inflammation-related pathways and could be investigated for the development of anti-inflammatory drugs [79]. Volatile chemicals possess a wide range of pharmacological actions, including antibacterial and anti-inflammatory activities, as well as effects on the central nervous system. Ongoing research into these substances and their interaction with biological systems continues to facilitate the development of new pharmaceutical products and therapeutic methods.

4. Conclusion

The current investigation unveiled the phytochemical makeup, as well as the anti-diabetic, anti-tyrosinase, and anti-inflammatory properties of the essential oil derived from *Ptychotis verticillata* Duby. The essential oil was found to be rich in γ-terpinene, carvacrol, and β-cymene, which are bioactive compounds that belong to the class of monoterpenes, and phenolic monoterpenes. These compounds have been reported to possess various biological, and pharmacological activities. The PVEO exhibited potent inhibitory effects on xanthine oxidase, tyrosinase, α-glucosidase, α-amylase, and lipoxygenase enzymes, which are involved in the pathogenesis of gout, diabetes, pigmentation disorders, and chronic inflammation, respectively. In addition, the PVEO had notable anti-inflammatory effects

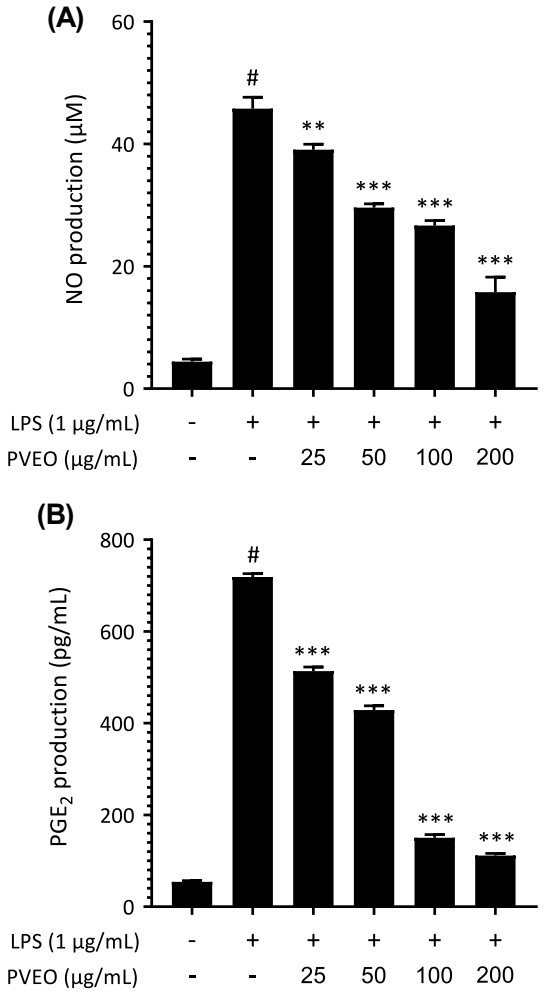

Fig. 3. The effect of PVEO on the synthesis of prostaglandin E2 (PGE2) and nitric oxide (NO) induced by LPS. (A) Following exposure to LPS (1 µg/mL), RAW 264.7 cells were cultured for a duration of 24 h with ascending concentrations (25–200 µg/mL) of PVEO. Utilizing the Griess reagent assay, the nitrite concentration in the culture medium was determined. (B) PGE_2 concentrations in the culture media were determined in accordance with the instructions provided in the materials using a commercially available assay kit. The data provided illustrates the mean and standard deviation of three separate experiments. A p-value less than 0.001 (#) indicates a statistically significant difference between the control group and the group treated with LPS alone. Significance levels of $p < 0.001$ (**) and $p < 0.01$ (***), respectively, denote substantial differences between the LPS-alone and PVEO treatment groups.

on the generation of NO and PGE_2 generated by LPS in RAW 264.7 cells. It dose-dependently decreased the levels of these inflammatory mediators without impacting the survival of the cells. These findings suggest that PVEO may mitigate inflammatory processes by modulating the expression of enzymes involved in the synthesis of NO and PGE_2. These results suggest that PVEO has a remarkable therapeutic potential for the management of these prevalent, and challenging health conditions, by modulating the enzymatic activities and preventing the accumulation of harmful metabolites or the excessive production of melanin. Moreover, the essential oil showed comparable to superior activity to the reference drugs allopurinol, acarbose, and quercetin, which are commonly used for the treatment of gout, diabetes, and inflammation, respectively. This indicates that the PVEO may offer a natural and effective alternative to these synthetic drugs, which may have adverse effects or limited efficacy. This study provides new insights into the pharmacological properties of *P. verticillata* and contributes to the valorization of this endemic plant as a source of bioactive compounds for the development of novel, and innovative agents that can address the unmet needs of patients suffering from gout, diabetes, pigmentation disorders, and chronic inflammation.

Data availability statement

Data will be available upon request from the corresponding author.

CRediT authorship contribution statement

Mohamed Taibi: Writing – original draft, Conceptualization. **Amine Elbouzidi:** Writing – original draft, Methodology. **Mounir Haddou:** Writing – original draft, Data curation. **Abdellah Baraich:** Writing – original draft, Formal analysis. **El Hassania Loukili:** Writing – original draft, Visualization. **Tarik Moubchir:** Writing – original draft, Resources. **Aimad Allali:** Writing – original draft, Investigation. **Amine khoulati:** Writing – original draft, Methodology. **Reda Bellaouchi:** Writing – original draft, Formal analysis. **Abdeslam Asehraou:** Writing – review & editing, Writing – original draft. **Mohamed Addi:** Writing – original draft, Data curation. **Ahmad Mohammad Salamatullah:** Writing – original draft, Supervision. **Mohammed Bourhia:** Writing – review & editing, Writing – original draft. **Farhan Siddique:** Writing – original draft, Validation. **Bouchra El Guerrouj:** Writing – original draft, Conceptualization. **Khalid Chaabane:** Writing – original draft, Visualization.

Antigout and antidiabetic activity: *In vitro inhibition of α-glucosidase, α-amylase, and xanthine oxidase*

table 2 presents the outcomes of investigations into the inhibitory impacts of essential oil derived from *Ptychotis verticillata* (PVEO) on three key enzymes: xanthine oxidase (E.C. 1.17.3.2), α-amylase (E.C. 3.2.1.1), and α-glucosidase (E.C. 3.2.1.20). These enzymes are involved in the metabolism of purines and carbohydrates, and their inhibition can help reduce the symptoms of gout and diabetes, respectively [49–51]. According to the table, PVEO exhibited significant inhibitory activity against all three enzymes in terms of IC_{50}, which is the concentration of a substance that inhibits 50% of the enzyme activity, and lower values indicate higher potency. PVEO was compared with two positive controls: allopurinol, a standard drug for gout that inhibits xanthine oxidase, and acarbose, a standard drug for diabetes that inhibits α-amylase and α-glucosidase. The findings indicate that PVEO had a comparable inhibitory impact on xanthine oxidase to allopurinol, with IC50 values of 25.33 ± 0.67 and 23.34 ± 0.09 μg/mL, respectively. Also, PVEO had a similar inhibitory effect on α-amylase, with IC_{50} values of 32.34 ± 0.78 μg/mL, while the essential oil had a relatively important inhibitory activity on α-glucosidase compared to the standard drug (acarbose, 65.41 ± 2.10 μg/mL) at 43.22 ± 3.03 μg/mL. Analysis of the chemical composition revealed the presence of various aromatic and terpene compounds, with major constituents such as γ-terpinene, β-cymene, carvacrol, and thymol. These compounds are recognized for their diverse biological properties, encompassing antioxidant, anti-inflammatory, and antidiabetic effects [52,53]. Notably, carvacrol and thymol, classified as phenolic monoterpenoids characterized by a hydroxyl group attached to a benzene ring [54,55], have demonstrated potent inhibitory effects on α-glucosidase, α-amylase, and xanthine oxidase in prior studies [56–59]. This investigation represents the first study to assess the anti-diabetic activity of this essential oil, making it difficult to compare with other previous studies. However, the results obtained in this study corroborate the findings of other research on essential oils rich in bioactive molecules. For example, the study conducted by Khedhri et al. (2023) on eucalyptus essential oils from Tunisia revealed that *E. leptophylla* and *E. brevifolia* exhibited remarkable inhibition of α-amylase, with an IC50 of 0.88 mg/mL [60]. Similarly, the study by Kochti et al (2024) confirmed the efficacy of essential oils rich in terpene molecules, highlighting the antienzymatic efficacy of *Callitris glaucophylla* essential oil [61].

The cumulative findings suggest that PVEO holds potential as a natural alternative for gout treatment and may emerge as a promising natural agent for diabetes management.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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