Advancement in Medicinal Plant Research
Vol. 14(1), pp. 1-8, February 2026
ISSN: 2354-2152
Full Length Research Paper

In vitro phytochemical analysis, antioxidant and enzymatic activities of Iranian *Callistemon citrinus* and *Platycladus orientalis*

Atefeh Bahmanzadagan[1*], Vahid Tavallali[2], Vahid Rowshan[1] and Seyed Mohammad Reza Habibian[1]

[1]Department of Natural Resources. Fars Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO),Shiraz, Iran.
[2]Department of Agriculture, Payame Noor University (PNU), Tehran, Iran.

Accepted 28 January, 2026

ABSTRACT

The compositional characterization of volatile constituents, evaluation of antioxidant potential, phenolic profiling, determination of total phenolic and total flavonoid contents, and assessment of antioxidant enzyme activities of the aerial parts of *Callistemon citrinus* and *Platycladus orientalis* were conducted with the aim of identifying new sources of natural bioactive compounds. Essential oils (EOs) and plant extracts were obtained using hydrodistillation and maceration techniques, respectively. In the essential oils, 1,8-cineole and α-pinene were identified as the dominant constituents of *C. citrinus* and *P. orientalis*, respectively. Both plant extracts exhibited pronounced antioxidant capacities. Gallic acid and sinapic acid were the major phenolic compounds in *C. citrinus*, whereas gallic acid and rutin predominated in *P. orientalis*. Enzymatic analyses revealed variable activities of catalase (CAT), peroxidase (POD), and polyphenol oxidase (PPO) in both species. Overall, the phytochemical findings highlight the potential of these plants as accessible and sustainable sources of natural bioactive agents, supporting their prospective applications in pharmaceutical, cosmetic, and dietary industries.

Keywords: Essential oil, phenolic compounds, antioxidant activity, *Callistemon citrinus*, *Platycladus orientalis*.

*Corresponding author. E-mail: bahmanzadegan2014@gmail.com; Tel: +98 7137204959; fax: +98 2172957276.

INTRODUCTION

The genus *Callistemon*, commonly known as bottlebrush, belongs to the family Myrtaceae and comprises more than 30 recognized species. Members of this genus are woody shrubs or small trees, typically ranging from 0.5 to 7 m in height, and are predominantly distributed in the humid tropical regions of Australia, South America, and tropical Asia. However, they are now cultivated worldwide (Mozaffarian, 2013; Safa et al., 2013). *Callistemon* species are utilized for various purposes, including essential oil production, reforestation, ornamental horticulture, windbreak establishment, and the rehabilitation of degraded lands (Saket et al., 2017). In Iran, these plants are widely cultivated as ornamental species in urban landscapes due to their visually striking inflorescences (Mozaffarian, 2013).

Phytochemical investigations of the genus have led to the identification of diverse secondary metabolites, notably C-methylated flavonoids, triterpenoids, and phloroglucinol derivatives (Shaha and Salunkhe, 2014). *Callistemon citrinus* (Curtis) Skeels, synonymously known as *C. lanceolatus*, is one of the most widely distributed species within the genus. It is particularly valued for its bright red, nectar-rich flower spikes, which attract birds (Larayetan et al., 2017). Comprehensive chemical analyses of the essential oil of *C. citrinus* from different geographical regions, including Australia, Egypt, Pakistan, India, and Réunion Island, have consistently identified 1,8-cineole (47.9–82.0%) as the major constituent, alongside other notable compounds such as α-pinene, β-pinene, myrcene, limonene, linalool, and methyl acetate.

Traditionally, *C. citrinus* has been used in the treatment of gastrointestinal disorders, microbial infections, pain, and respiratory conditions such as bronchitis and cough. Moreover, its essential oil has demonstrated antimicrobial activity, while leaf extracts have shown anti-inflammatory and antifungal properties (Khammassi et al., 2022; Larayetan et al., 2017).

Platycladus orientalis (L.) Franco, commonly known in Iran as “sarv-e khomrerii” or “nosh,” is an evergreen conifer indigenous to the region and widely cultivated worldwide for ornamental and medicinal purposes (Larayetan et al., 2014). This monoecious tree or shrub typically reaches heights of 10–60 feet and is characterized by flattened shoots bearing scale-like leaves with resin-secreting glands. In traditional medicine, topical preparations of *P. orientalis* have been used to treat skin infections such as scabies and impetigo. Phytochemical studies of various plant parts have identified a wide range of monoterpenes, sesquiterpenes, and flavonoids (Yan-hua et al., 2006).

Despite their widespread utilization and reported biological activities, comprehensive information regarding the volatile oil composition, phenolic profiles, antioxidant properties, and enzymatic activities of the aerial parts of *C. citrinus* and *P. orientalis* remains limited. Therefore, the present study aims to address this knowledge gap by investigating the essential oil constituents, phenolic composition, antioxidant potential, and antioxidant enzyme activities of these species, with particular emphasis on samples collected from Fars Province, southern Iran.

MATERIALS AND METHODS

Plant material

The aerial parts of *Callistemon citrinus* (stems, leaves, and flowers) and *Platycladus orientalis* (stems, leaves, flowers, and fruits) were collected in May 2024 from cultivated plants grown in the research gardens of the Fars Agricultural and Natural Resources Research Center (FANRC), located in Fars Province, southern Iran. The sampling site was georeferenced at 52°35′13″ E longitude and 29°34′33″ N latitude, at an altitude of 1510 m above sea level. Plant identification and authentication were performed by taxonomists at the FANRC Herbarium in Shiraz, where voucher specimens were deposited under accession numbers 5482 (*C. citrinus*) and 6354 (*P. orientalis*).

Essential oil extraction and analysis

Following collection, plant materials were shade-dried under ambient conditions. Essential oils (EOs) were extracted from 100 g of dried aerial parts by hydrodistillation for 3 h using an all-glass Clevenger-type apparatus, following the British Pharmacopoeia (1988) method. The obtained oils were dried over anhydrous sodium sulfate and stored at 4 °C until analysis. All extractions were performed in quadruplicate.

Gas chromatography (GC) analysis was carried out using an Agilent 7890A gas chromatograph equipped with a flame ionization detector (FID). Separation was achieved on an HP-5 fused silica capillary column (30 m × 0.32 mm i.d., 0.25 µm film thickness, 5% phenylmethylpolysiloxane). The oven temperature was set at 60 °C, injector temperature at 250 °C, and detector temperature at 280 °C. Nitrogen was used as the carrier gas at a flow rate of 1 mL/min, with an injection volume of 0.2 µL and a split ratio of 1:50.

Gas chromatography–mass spectrometry (GC–MS) analysis was performed using an Agilent 5975-C system equipped with an HP-5MS column and a mass selective detector. The injection volume was 0.1 µL. Identification of volatile constituents was based on comparison of their retention indices with those of n-alkanes (C8–C25) analyzed under identical conditions, as well as comparison of mass spectra with commercial spectral libraries, reference standards, and published data (Adams, 2007). Relative component percentages were calculated from FID peak areas without correction factors.

Preparation of crude extracts

Polyphenolic compounds were extracted using a modified maceration method based on Justesen et al. (1998). Briefly, 5.0 g of shade-dried aerial plant material from each species was immersed in 50 mL of 100% methanol (1:10, w/v) and kept in the dark at room temperature for 24 h. The extracts were filtered through 0.22 µm membrane filters prior to HPLC analysis. Filtrates were concentrated under reduced pressure at 40 °C using a rotary evaporator and stored for subsequent antioxidant activity, total phenolic content (TPC), and total flavonoid content (TFC) analyses.

HPLC analysis of phenolic compounds

Eighteen phenolic standards (Merck, Darmstadt, Germany) were used for compound identification and quantification. Analyses were conducted using an Agilent 1200 series HPLC system equipped with a Zorbax Eclipse XDB-C18 column (4.6 × 150 mm, 5 µm). A 20 µL aliquot of each extract was injected, and the column temperature was maintained at 30 °C.

Separation was achieved using a gradient elution system consisting of methanol and 1% formic acid. The gradient program was as follows: 10:90 (v/v) at 0 min, 25:75 at 10 min, 60:40 at 20 min, and 70:30 at 30 min, followed by isocratic elution until 40 min. The flow rate was set at 1 mL/min. Detection was performed using a

photodiode array (PDA) detector at 280 and 320 nm. All analyses were conducted in quadruplicate. Identification and quantification were based on retention times, UV spectra comparison with authentic standards, and external calibration curves (Bahmanzadegan et al., 2019).

Determination of Total Phenolic Content (TPC)

TPC was determined using the Folin–Ciocalteu method. Briefly, 100 µL of extract was mixed with 200 µL of 50% Folin–Ciocalteu reagent and 1000 µL of 20% sodium carbonate solution. After vortexing for 15 s, the mixture was incubated in the dark at room temperature for 30 min. Absorbance was measured at 765 nm using a UV–Vis spectrophotometer. Gallic acid was used as the calibration standard, and results were expressed as mg gallic acid equivalents (GAE) per gram of extract. All measurements were performed in quadruplicate (Bahmanzadegan et al., 2022).

Determination of Total Flavonoid Content (TFC)

TFC was determined by mixing 0.5 mL of extract with 0.5 mL of 2% $AlCl_3$ methanolic solution. The reaction mixture was incubated at room temperature for 60 min, after which absorbance was recorded at 510 nm. Quercetin was used as the reference standard, and results were expressed as mg quercetin equivalents (QE) per gram of extract. All analyses were carried out in quadruplicate (Bahmanzadegan et al., 2022).

Antioxidant activity assay

Free radical scavenging activity was evaluated using the DPPH* (2,2-diphenyl-1-picrylhydrazyl) assay following the method of Bahmanzadegan et al. (2022). A 100 µL aliquot of each extract was added to 5 mL of 0.004% DPPH* methanolic solution. The mixture was incubated in the dark at room temperature for 30 min, and absorbance was measured at 517 nm against a blank. All experiments were performed in quadruplicate. Radical scavenging activity was calculated using the following equation:

$$\text{Scavenging (\%)} = (1 - A_{517}\text{sample} / A_{517}\text{blank}) \times 100$$

Evaluation of antioxidant enzyme activities

Preparation of Crude Enzyme Extracts (CEE)

Fresh aerial tissues (0.5 g) were homogenized in 2 mL of ice-cold 50 mM potassium phosphate buffer (pH 7.0) containing 2 mM Na-EDTA and 1% (w/v) polyvinylpolypyrrolidone (PVP). The homogenate was centrifuged at 13,000 rpm for 10 min at 4 °C. The supernatant was collected as the crude enzyme extract (CEE) and stored at −80 °C until analysis (Ozden et al., 2009).

Peroxidase (POD) assay

POD activity was determined using a modified method of MacAdam and Nelson Sharp (1992). The reaction mixture (2 mL) consisted of 0.1 mL CEE, 1.7 mL of 50 mM potassium phosphate buffer (pH 7.0), 0.15 mL of 40 mM guaiacol, and 0.05 mL of 40 mM hydrogen peroxide. Changes in absorbance were recorded at 470 nm at 10-s intervals. Enzyme activity was calculated using an extinction coefficient of 26.6 mM^{-1} cm^{-1} for guaiacol. All assays were performed in quadruplicate.

Polyphenol Oxidase (PPO) assay

PPO activity was measured using catechol as substrate according to the modified method of Ghanati et al. (2002). The reaction mixture contained 0.3 mL of 600 mM catechol, 2.3 mL of 100 mM phosphate buffer (pH 7.0), and 0.1 mL of CEE. Absorbance was measured at 425 nm immediately and after 30 min of incubation. PPO activity was calculated using the extinction coefficient of catechol (2.2×10^3 mM^{-1} cm^{-1}). All measurements were conducted in quadruplicate.

Catalase (CAT) assay

Catalase activity was assayed following the method of Çakmak and Horst (1991), with minor modifications. The reaction mixture (3 mL) contained 2.8 mL of 50 mM potassium phosphate buffer (pH 7.0), 0.1 mL of 0.1 M H_2O_2, and 0.1 mL of CEE. The decrease in absorbance was monitored at 240 nm for 1 min. CAT activity was calculated using an extinction coefficient of 43.6 mM^{-1} cm^{-1} for H_2O_2. Each sample was analyzed in quadruplicate.

RESULTS

The essential oils (EOs) isolated from *Callistemon citrinus* and *Platycladus orientalis* were pale yellow liquids, with yields of 1.42% and 1.95%, respectively. Qualitative and quantitative analyses of the EOs were performed using gas chromatography coupled with a flame ionization detector (GC–FID) and gas chromatography–mass spectrometry (GC–MS). A total of 41 compounds were identified in *C. citrinus* EO,

representing 98.0% of the total oil composition, whereas 48 compounds accounting for 98.9% were identified in *P. orientalis* EO (Table 1).

The major constituents of *C. citrinus* EO were 1,8-cineole (47.7%), α-pinene (27.6%), p-cymene (5.0%), α-terpineol (3.6%), flavesone (2.5%), and α-phellandrene (2.3%). In contrast, *P. orientalis* EO was dominated by α-pinene (46.0%), δ-3-carene (21.1%), sabinene (6.1%), terpinolene (4.8%), myrcene (3.3%), and β-phellandrene (2.3%). Based on chemical classification, oxygenated monoterpenes were predominant in *C. citrinus*, whereas monoterpene hydrocarbons constituted the major fraction in *P. orientalis* EO.

High-performance liquid chromatography (HPLC) analysis enabled the identification and quantification of 18 phenolic compounds in total, with 10 compounds detected in *C. citrinus* and 8 in *P. orientalis* extracts (Table 2). Identified phenolics included catechin, quercetin, sinapic acid, gallic acid, hesperetin, p-coumaric acid, hesperidin, rutin, naringenin, and myricetin. Gallic acid and sinapic acid were the predominant phenolic compounds in *C. citrinus*, whereas gallic acid and rutin were the dominant constituents in *P. orientalis*.

Table 1. Essential oil composition of *Callistemon citrinus* and *Platycladus orientalis*.

Compound	RI [a]	% [b]	
	Callistemon citrinus	***Platycladus orientalis***
Propyl butanoate	890	t[c]	-
Tricyclene	920	-	t
α-Thujene	926	1.0±0.01	1.4±0.01
α-Pinene	933	27.6±0.2	46.0±0.3
α-Fenchene	953	-	1.3±0.01
Camphene	954	t	-
Sabinene	975	t	6.1±0.2
β-Pinene	979	0.9±0.1	1.3±0.1
Myrcene	990	0.4±0.01	3.3±0.1
α-Phellandrene	1001	2.3±0.1	0.6±0.01
δ-3-Carene	1004	0.1±0.01	21.1±0.2
α-Terpinene	1020	0.1±0.01	0.6±0.01
p-Cymene	1025	5.0±0.1	0.3±0.01
Limonene	1029	1.2±0.01	0.8±0.01
β-Phellandrene	1030	-	2.3±0.1
1,8-Cineole	1032	47.7±0.2	t
(*Z*)-β-Ocimene	1036	-	t
(*E*)-β-Ocimene	1050	0.2±0.01	t
γ-Terpinene	1060	0.6±0.01	1.0±0.02
cis-Sabinene hydrate	1073	-	t
Terpinolene	1089	0.4±0.01	4.8±0.1
Linalool	1099	0.3±0.01	-
n-Nonanal	1107	t	-
endo-Fenchol	1117	t	-
cis-*p*-Menth-2-en-1-ol	1121	t	t
α-Campholenal	1128	t	-
trans-Pinocarveol	1138	t	-
trans-*p*-Menth-2-en-1-ol	1139	-	t
Camphor	1145	-	t
Borneol	1169	-	t
Terpinen-4-ol	1179	0.4±0.01	1.2±0.1
α-Terpineol	1190	3.6±0.1	0.1±0.01
cis-Piperitol	1198	-	t
trans-Piperitol	1208	-	t
Nerol	1228	t	t
Geraniol	1259	0.2±0.01	-
Geranial	1269	0.4±0.01	-
Bornyl acetate	1285	-	0.4±0.01
Thymol	1290	-	-
Carvacrol	1298		-
Terpinen-4-ol acetate	1299	-	t
δ-Elemene	1339	-	0.2±0.01

Table 1. Continues.

α-Terpinyl acetate	1349	-	0.6±0.1
Eugenol	1360	0.3±0.01	-
Neryl acetate	1363	-	t
α-Copaene	1377	0.2±0.01	-
Geranyl acetate	1384	t	t
β-Elemene	1393	-	0.2±0.01
Phenyl ethyl isobutanoate	1395	0.1±0.01	-
(*Z*)-Caryophyllene	1410	-	0.4±0.1
(*E*)-Caryophyllene	1422	t	1.5±0.1
cis-Thujopsene	1428	-	0.2±0.1
Aromadendrene	1441	t	-
α-Humulene	1455	0.1±0.01	0.9±0.1
allo-Aromadendrene	1461	t	-
Germacrene D	1483	-	0.4±0.1
δ-Cadinene	1523	-	t
Flavesone	1545	2.5±0.2	-
Elemol	1550	-	t
Germacrene B	1559	-	t
Spathulenol	1577	0.6±0.1	t
Caryophyllene oxide	1585	0.1±0.01	-
Cedrol	1596	-	1.8±0.1
epi-Cedrol	1613	-	0.1±0.01
1,10-di-epi-Cubenol	1619	-	t
Leptospermone	1620	0.5±0.01	-
iso-Leptospermone	1625	1.0±0.01	-
1-epi-Cubenol	1629	-	t
(*Z,Z*)-2,6-Farnesol	1719	0.2±0.01	-
Monoterpene hydrocarbons		39.8	90.9
Oxygenated Monoterpenes		55.5	2.3
Sesquiterpene hydrocarbons		0.3	3.8
Oxygenated Sesquiterpenes		2.4	1.9
Total		**98.0**	**98.9**

[a]RI,Retention indices relative to C_8–C_{25} n-alkanes on the HP-5 column; [b] % of compounds; [c] t, trace <0.1%.

Antioxidant activity of the methanolic extracts was evaluated using the DPPH radical scavenging assay, which is based on the reduction of the purple DPPH radical to a yellow-colored product upon interaction with antioxidants (Ozturk et al., 2007; Akowuah et al., 2005). The IC_{50} values obtained were 84.52 µg/mL for *C. citrinus* and 136.60 µg/mL for *P. orientalis*, indicating strong radical scavenging capacity. Both extracts exhibited higher antioxidant activity than the reference standard α-tocopherol (IC_{50} = 184.15 µg/mL).

Phenolic compounds contribute to antioxidant activity through redox mechanisms, including electron donation and free radical neutralization (Stoilova et al., 2007). The total phenolic content (TPC) of *C. citrinus* extract was 828.19 mg gallic acid equivalents (GAE)/g extract, which exceeded previously reported values for samples from India and Turkey (261 and 24.98 mg GAE/g, respectively). Similarly, *P. orientalis* exhibited a high TPC value of 829.10 mg GAE/g extract, markedly greater than those reported for ethanolic extracts from Tunisia (61.44 mg GAE/g; Khammassi et al., 2022).

Total flavonoid content (TFC) values were 72.36 mg quercetin equivalents (QE)/g extract for *C. citrinus* and 136.11 mg QE/g extract for *P. orientalis*. The presence of flavonoids such as hesperidin, quercetin, rutin, and related compounds likely contributes to the observed antioxidant activities through mechanisms including metal chelation and inhibition of lipid peroxidation (Zaragoza et al., 2008).

Enzymatic activity assays (Table 2) revealed substantial catalase (CAT) activity in both species, highlighting their capacity for hydrogen peroxide detoxification. *C. citrinus* exhibited higher polyphenol oxidase (PPO) activity, which may be associated with enhanced defense responses against biotic stress (Zhang and Sun, 2021). Additionally, peroxidase (POD) activity was elevated in *C. citrinus*, suggesting a role in lignin biosynthesis and oxidative stress tolerance (Afzali et al., 2023). The coordinated activity of these antioxidant enzymes contributes to improved physiological

Table 2. Phenolic compounds (PCs), antioxidant activity and total phenol (TPC), flavonoid (TFC) contents and antioxidant enzyme activities of *Callistemon citrinus* and *Platycladus orientalis.*

PCs	Amount [a]	
	C. citrinus	***P. orientalis***
Catechin	3.86±0.20	0.21±0.01
Carvacrol	nd[b]	nd
Caffeic acid	nd	nd
Quercetin	0.91±0.01	0.13±0.01
Rosmarinic acid	nd	nd
Sinapic acid	6.01±0.10	nd
Gallic acid	20.52±0.11	7.24±.010
Chloregenic acid	nd	0.10
Hesperetin	5.50±0.01	nd
Vanillin	nd	nd
p-Coumaric acid	0.59±0.01	0.05±0.01
trans-Ferulic acid	nd	nd
Hesperidin	2.76±0.10	nd
Rutin	3.74±0.20	6.57±0.24
Coumarin	nd	nd
Eugenol	nd	nd
Naringenin	2.45±0.12	1.51±0.10
Myricetin	4.12±0.20	5.64±0.24
Antioxidant activity by DPPH (µg/ml)	84.52±1.15	136.60±2.45
TPC (mg Ga/g extract)	828.19±2.12	829.10±2.30
TFC (mg Qu/g extract)	72.36±1.14	136.11±1.74
Catalase (unit g^{-1} FW[c])	560±2.32	354±1.53
Polyphenol oxidase (unit g^{-1} FW)	34±1.35	12.6 ±1.10
Peroxidase (unit g^{-1} FW)	54 ±1.63	38.34±1.14

[a]Calculated mean amount of the polyphenol (mg/g) based on the weight of the ground dry plant; [b]not detected; [c]fresh weight.

adaptability and stress resilience in both plants (Gassad and Fajer, 2022).

DISCUSSION

Previous studies on the phytochemical composition of *Callistemon citrinus* and *Platycladus orientalis* remain relatively limited; however, available reports are largely consistent with the findings of the present study. For instance, essential oil extracted from *C. citrinus* leaves collected in South Africa was reported to contain high levels of 1,8-cineole (48.98%) and α-pinene (20.02%) (Larayetan et al., 2017), which closely aligns with the dominant constituents identified in the current investigation. In contrast, essential oil from *P. orientalis* collected in Kogi State, Nigeria, was characterized by lower α-pinene content (15.9%) and higher proportions of α-caryophyllene (10.4%) and trans-β-ocimene (8.7%) (Larayetan et al., 2014), indicating notable geographical variation in volatile profiles.

Phytochemical investigations of *C. citrinus* have revealed the presence of diverse secondary metabolites, including triterpenoids, steroids, carbohydrates, tannins, and flavonoids. Within the *Callistemon* genus, triterpenoids and steroids are commonly reported in leaves, seeds, and stem bark, while flavonoids and phenolic acids contribute substantially to biological activity. Several phenolic and flavonoid compounds isolated from related *Callistemon* species have demonstrated cytotoxic activity against cancer cell lines in vitro, although the specific compounds and their efficacy depend on the plant tissue examined and extraction method employed.

Consistent with previous reports, *C. citrinus* exhibited strong antioxidant capacity in the present study. Earlier investigations demonstrated that methanolic extracts of *C. citrinus* possess substantial DPPH free radical scavenging activity, while essential oils obtained from leaves and flowers showed antioxidant effects at concentrations of 1.49 and 1.13 mg/mL, respectively (Larayetan et al., 2017). The pronounced antioxidant activity observed here may be attributed, in part, to the high content of oxygenated monoterpenes and phenolic compounds, which are known to enhance endogenous antioxidant defense systems. Notably, terpenes present in *C. citrinus* have been reported to induce antioxidant enzymes, a mechanism considered relevant to cancer chemoprevention and cellular protection against oxidative stress.

Enzymatic activity results further support the biological relevance of *C. citrinus*. Elevated activities of catalase, peroxidase, and polyphenol oxidase observed in this species suggest a coordinated enzymatic response to oxidative challenges. Previous studies have also shown that extracts of *C. citrinus* can modulate enzymes involved in inflammatory pathways, reinforcing its potential role in managing oxidative stress–related disorders and inflammation. Collectively, these findings position *C. citrinus* as a promising source of bioactive compounds with therapeutic relevance.

Among the major phenolic compounds identified, gallic acid, classified as a hydrolyzable tannin, exhibits a broad spectrum of biological activities, including anticancer, antimicrobial, and anti-inflammatory effects (Yang et al., 2020). Sinapic acid, a predominant hydroxycinnamic acid detected in *C. citrinus*, has been reported to possess stronger antioxidant activity than ferulic acid and is increasingly valued for applications in food preservation and cosmetic formulations (Bahmanzadegan et al., 2023). In *P. orientalis*, rutin a glycoside composed of quercetin and rutinose, was among the major phenolics and is well recognized for its neuroprotective and cardioprotective properties (Ganeshpurkar and Saluia, 2017).

Similar to *C. citrinus*, *P. orientalis* has been extensively investigated for its phytochemical constituents. Essential oils and extracts of this species typically contain monoterpenes such as α-pinene, β-pinene, sabinene, limonene, γ-terpinene, camphene, β-phellandrene, 1,8-cineole, α-terpinene, p-cymene, α-terpineol, and terpinolene. In addition to terpenoids, flavonoids and phenolic compounds have been consistently reported from various plant parts. These constituents are believed to underlie the wide range of pharmacological activities attributed to *P. orientalis*, including antioxidant and anti-inflammatory effects documented in earlier studies.

Overall, the results of this study demonstrate that extracts of *C. citrinus* and *P. orientalis* are rich in phenolic compounds and flavonoids that significantly contribute to antioxidant activity by scavenging free radicals. The antioxidant efficacy of these compounds is closely linked to their chemical structure, particularly the presence and number of functional groups such as hydroxyl (–OH) and amino ($–NH_2$) moieties. In polyphenolic compounds, specific structural features have also been associated with the modulation of enzyme activity, suggesting a complex relationship between molecular structure, antioxidant potential, and enzymatic regulation. This multifaceted bioactivity underscores the value of these species as promising sources of natural antioxidants with potential applications in pharmaceutical, nutraceutical, and cosmetic industries.

CONCLUSION

This study provides a comprehensive evaluation of the chemical composition and bioactive potential of *Callistemon citrinus* and *Platycladus orientalis*. The strong positive correlation observed between total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity ($r = 0.998$, $p < 0.01$) underscores the functional importance of phenolic and flavonoid compounds in mediating antioxidant capacity. Both species demonstrated considerable potential as rich and sustainable sources of bioactive constituents, particularly oxygenated monoterpenes and polyphenols, which may be valuable for nutraceutical and pharmaceutical applications. Further investigations are warranted to elucidate the molecular mechanisms underlying their bioactivities and to explore their incorporation into health-promoting formulations.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support provided by the Fars Research Center for Agriculture and Natural Resources, Shiraz, Iran.

Declaration of interest

The authors declare no competing financial or personal interests that could have influenced the work reported in this paper.

REFERENCES

Adams RP, **2007**. Identification of essential oil components by gas chromatography/mass spectroscopy. Allured Publishing Corporation, Illinois, 1-804.

Akowuah GA, Ismail Z, Norhayati I, Sadikun A, **2005**. The effects of different extraction solvents of varying polarities on polyphenols of Orthosiphon stamineus and evaluation of the free radical-scavenging activity. Food Chem, 93: 311-317.

Afzali SF, Sadeghi H, Taban A, **2023**. A comprehensive model for predicting the development of defense system of *Capparis spinosa* L.: a novel approach to assess the physiological indices. Sci Rep, 13: 12413.

Bahmanzadegan A, Rowshan V, Zareiyan F, Hatami A, **2019**. *Lagoecia cuminoides* L., its antioxidant activity and polyphenolic constituents from Iran. Nat Prod Res, 33: 2376-2378.

Bahmanzadegan A, Tavallali H, Tavallali V, Karimi MA, **2022**. Variations in biochemical characteristics of *Zataria multiflora* in response to foliar application of zinc nano complex formed on pomace extract of *Punica granatum*. Ind Crops Prod, 187:115369.

Bahmanzadegan A, Zareiyan F, Rowshan V, Hatami A, Habibian MR, **2023**. Chemical composition and antioxidant activity of essential oil from *Lavandula sublepidota*. Chem Nat Compd, 59: 578-581.

British pharmacopoeia, **1988**. 1988 British pharmacopoeia. London: HMSO. 2: 137–138.

Cakmak I, **Horst** W, **1991**. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tip of soybean (*Glysin max*). Plant Physiol, 83: 463-468.

Ganeshpurkar A, **Saluia** AK, **2017**. The pharmacological potential of rutin. Saudi Pharm J, 25: 149-164.

Gassad SH, **Fajer** AN, **2022**. Comparison Between Effective Compounds Between Peel and Seeds of *Capparis Spinosa*. Int J Health Sci, 6: 11012-11021.

Ghanati F, Morita A, Yokota H, **2002**. Induction of suberin and increase

of liginin content by excess boron in tobacco cell. Soil Sci Plant Nutr, 48: 357–364.
Justesen U, Knuthsen P, Leth T, **1998**. Quantitative analysis of flavonols, flavones and flavanons in fruits, vegetables and beverages by HPLC with photodiode array and mass spectrometric detection. Leth J Chromatogr A, 799: 101-110.
Khammassi M, Ayed RB, Khedhri S, Souihi M, Hanana M, Amri I, Hamrouni L, **2022**. Crude extracts and essential oil of *Platycladus orientalis* (L.) Franco: a source of phenolics with antioxidant and antibacterial potential as assessed through a chemometric approach. Turk J Agric For, 46: 477-487.
Larayetan RA, Okoh OO, Sadimenko A, Oko AI, **2017**. Terpene constituents of the aerial parts, phenolic content, antibacterial potential, free radical scavenging and antioxidant activity of *Callistemon citrinus* (*Curtis*) Skeels (Myrtaceae) from Eastern Cape Province of South Africa. BMC Complement Altern Med, 17: 292.
Larayetan RA, Sodamade A, Owonikoko AD, **2014**. Volatile constituents & phytochemical studies of *Platycladus orientalis* grown in north central Nigeria. IOSR J Appl Chem, 7: 243-247.
MacAdam JW, **Nelson Sharp** CJ, **1992**. Peroxidase activity in the leaf elongation zone of tall fescue. Plant Physiol, 99: 872-878.
Mozaffarian V, **2013**. Identification of Medicinal and Aromatic Plants of Iran. Tehran: Farhang Moaser Press.
Ozden M, Demirel U, Kahraman A, **2009**. Effects of proline on antioxidant system in leaves of grapevine (*Vitis vinifera* L.) exposed to oxidative stress by H_2O_2. Sci Hortic, 119: 163-168.
Ozturk M, Ozturk FA, Duru ME, Topcu G, **2007**. Antioxidant activity of stem and root extracts of Rhubarb (*Rheum ribes*): An edible medicinal plant. Food Chem, 103: 623-630.
Safa O, Soltanipoor MA, Rastegar S, Kazemi M, Nourbakhsh Dehkordi Kh, Ghannadi A, **2013**. An ethnobotanical survey on hormozgan province, Iran. Avicenna J Phytomed, 3: 64-81.
Saket Kh, Feyzabadi Z, Khodabakhsh M, Hamedi Sh, **2017**. Comparing epilepsy treatment in Iranian traditional medicine and modern medicine. Hist Med J, 30: 27-53.
Shaha A, **Salunkhe** VR, **2014**. Development and validation of a high performance thin layer chromatographic method for determination of 1,8-cineole in *Callistemon citrinus*. Pharm Res, 6: 143.
Stoilova I, Krastanov A, Stoyanova A, Denev P, Gargova PS, **2007**. Antioxidant Activity of a Ginger Extract (*Zingiber officinale*). Food Chem, 102: 764-770.
Yan-hua L, Zhi-yong L, Zheng-tao W, **2006**. Quality evaluation of *Platycladus orientalis* (L) Franco through simultaneous determination of four bioactive Flavonoids by high performance liquid chromatography. J Pharm Biomed Anal, 41: 1186-1190.
Yang K, Zhang L, Liao P, Xiao Z, Zhang F, Sindaye D, Xin Z, Tan Ch, Deng J, Yin Y, Deng B, **2020**. Impact of gallic acid on gut health: focus on the gut microbiome, immune response, and mechanisms of action. Front Immunol, 11: 580208.
Zaragoza MC, Lopez D, Saiz MP, Poquet M, Perez J, Puig-Parellada P, Marmol F, Simonetti P, Gardana C, Lerat Y, Burtin P, Inisan C, Rousseau I, Besnard M, Mitjavila MT, **2008**. Toxicity and antioxidant activity in vitro and in vivo of two *Fucus vesiculosus* extracts. J Agric Food Chem, 56: 7773-7780.
Zhang J, **Sun** X, **2021**. Recent advances in polyphenol oxidase-mediated plant stress responses. Phytochemistry, 181: 112588.

Citation: Bahmanzadagan A, Tavallali V, Rowshan V, Habibian SMR, **2026**. *In vitro* phytochemical analysis, antioxidant and enzymatic activities of Iranian *Callistemon citrinus* and *Platycladus orientalis*. Adv Med Plant Res, 14(1): 1-8.