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Original Article

Antioxidant and antifungal properties of methanolic and aqueous extracts of *Satureja hortensis* and *Cinnamomum zeylanicum*

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Abstract

Plant-derived compounds possess robust antioxidant capabilities and exhibit notable antimicrobial activities against diverse pathogens. This study investigates the antioxidant activities of methanolic and aqueous extracts of *Satureja hortensis* (*S. hortensis*) and *Cinnamomum zeylanicum* (*C. zeylanicum*). The minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) of the extracts were also evaluated. The antifungal activity of methanolic extract of *S. hortensis* (MIC 1.25 mg/mL) was higher than that of the aqueous extract (MIC 5 mg/mL) on *P. chrysogenum*. For *Candida* species, especially *C. albicans*, MIC and MFC of methanolic extract were lower (MIC 0.31 mg/mL, MFC 1.25 mg/mL) than aqueous extract. The methanolic extract of *C. zeylanicum* exhibited a MIC of 0.31 mg/mL and MFC of 0.62 mg/mL against *P. chrysogenum*, outperforming the aqueous extract (MIC 0.62 mg/mL, MFC 1.25 mg/mL). Similarly, methanolic extracts showed markedly lower MIC values than the aqueous extracts against *Candida* species. In antioxidant activity of methanolic and aqueous extracts of both plants, ABTS values were consistently higher than DPPH results, likely due to ABTS's broader sensitivity to hydrophilic and lipophilic antioxidants. In the DPPH assay, the methanolic extract of *C. zeylanicum* exhibited the highest antioxidant activity with a scavenging percentage of 89.16 ± 0.6%. In the ABTS method, the methanolic extract of *C. zeylanicum* exhibited highest antioxidant activity, with a scavenging percentage of 97.8 ± 0.1%. The results of this study indicate that *S. hortensis* and *C. zeylanicum* extracts are promising natural antifungals and antioxidants.

Keywords: Antioxidant, *Candida*, *Cinnamomum zeylanicum*, Extract, *Satureja hortensis*, *Penicillium chrysogenum*

Introduction

Lipid oxidation, a chemical process characterized by the degradation of unsaturated fatty acids or their derivatives, leads to the formation of undesirable compounds that negatively affect the flavor and quality of food products, commonly recognized as rancidity. Beyond its impact on taste, rancidity diminishes the nutritional value of food (Geng et al, 2023). Antioxidants are frequently used to stabilize fats and prevent oxidative damage. However, the traditional use of synthetic antioxidants for food preservation has raised concerns owing to their potential toxicity and carcinogenic effects. Consequently, there is a growing demand for safer, naturally derived alternatives (Gutiérrez-del-Río et al., 2021; Ji et al., 2024).

Medicinal plants have been used for centuries as a valuable source of bioactive compounds with diverse pharmacological properties (Angane et al., 2022; Chávez-Delgado & Jacobo-Velázquez, 2023). Plant extracts are rich in bioactive compounds, notably polyphenols, which have emerged as promising

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natural additives in meat products. These compounds exhibit significant antioxidant properties, effectively slowing oxidative processes and extending the shelf life of meat products (Sah et al., 2014).

The genus *Satureja* L., family *Lamiaceae*, includes over 30 species of aromatic herbs and shrubs. These plants are predominantly distributed across the Mediterranean region. The antifungal properties of *Satureja hortensis* essential oil (EO) have been studied (Fierascu et al., 2018). Its major components, thymol and carvacrol, disrupt fungal cell membranes and inhibit biofilm formation. This activity is attributed to structural deformation and shrinkage of fungal cells (Sharifzadeh et al., 2016). *Satureja* species are rich in phenolic compounds such as rosmarinic acid and flavonoids, which exhibit strong antioxidant properties. These compounds act as free radical scavengers, protecting cells from oxidative stress (Ejaz et al., 2023; Güllüce et al., 2003).

Extracts and EOs derived from *Cinnamomum* species exhibit remarkable antioxidant properties, primarily attributed to their high content of phenolic and polyphenolic compounds such as cinnamaldehyde (Davoudi & Ramazani, 2024). *Cinnamomum* species are also well known for their broad-spectrum antifungal properties. EOs from *Cinnamomum zeylanicum* bark have demonstrated significant efficacy against fungal pathogens such as *C. albicans* (Valizadeh et al., 2015). These antifungal effects are primarily attributed to bioactive compounds such as cinnamaldehyde and eugenol, which disrupt fungal cell membranes and inhibit biofilm formation (Yang et al., 2012). Pathogenic molds, including species from the *Penicillium*, *Aspergillus*, and *Fusarium* genera, are major contributors to food spoilage and foodborne illnesses. These molds grow on various food products, particularly those with high moisture content, and produce visible changes, such as texture alteration, development of unpleasant odors, and discoloration. Beyond their impact on food quality, these molds pose significant health risks owing to their ability to produce mycotoxins (Lahlali et al., 2022; Pandey et al., 2021).

Candida species are the primary causes of fungal infections in humans. Although *C. albicans* is the most common species associated with diseases, *C. tropicalis*, *Nakaseomyces glabratus* (*C. glabrata)*, and *Pichia kudriavzevii* (*C. krusei)* are also frequently observed (Vieira et al., 2018).

The emergence of antifungal resistance in fungal pathogens underscore the urgent need for novel therapeutic agents. Bioactive compounds are natural alternatives for combating fungal infections and are used as food preservatives. The antifungal and antioxidant activities of *Satureja* and *Cinnamomum* extracts make them valuable for applications in medicine and food preservation. The choice of the extraction method significantly influences the yield and bioactivity of plant-derived compounds. In addition to the extraction method, the geographic origin of plants profoundly affects their chemical composition (Tian et al., 2023). Despite studies on antimicrobial and antioxidant properties of *S. hortensis*, no research has been conducted on the antioxidant or antifungal activities of methanolic and aqueous extracts of *S. hortensis* collected from Ardabil Province, northwestern Iran. Thus, this study was designed to assess the in vitro antioxidant activities of the methanolic and aqueous extracts of *S. hortensis* and *C. zeylanicum* using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays. Minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) of these extracts against *P. chrysogenum* and *Candida* spp. were determined using broth microdilution method.

Materials and Methods

Materials

All the chemicals used were of analytical grade. Potassium persulfate, DPPH, ABTS, butylated hydroxytoluene (BHT), Roswell Park Memorial Institute medium (RPMI 1640), methanol, and morpholine propanesulfonic acid (MOPS) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Sabouraud dextrose agar (SDA) was supplied by Biolife, Italy. Fungal species used in this study were obtained from the Mycology Laboratory of the Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.

Plant materials

Aerial parts of *S. hortensis* were collected from the slopes of Mount Sabalan, located in Ardabil Province, northwestern Iran. The plant material was air-dried in a shaded area at room temperature (25 °C) for 14 d to preserve its bioactive components. The botanical identification of the plant was confirmed by the Herbarium of the Faculty of Pharmacy at the University of Tabriz, Tabriz, Iran. *C. zeylanicum* powder was obtained from a local market in Tabriz, East Azerbaijan Province, Iran.

Preparation of methanolic and aqueous extracts of plants

Aqueous and methanolic extracts of *S. hortensis* and *C. zeylanicum* were prepared as following: The dried aerial parts of *S. hortensis* were pulverized using a grinder (Dintok, Germany). To prepare methanolic and aqueous extracts, 25 g of plant powder was mixed separately with 225 mL of methanol (99.5%) and distilled water, respectively. The mixtures were shaken in an incubator (Jeiotech, South Korea) at room temperature for 24 h. Following incubation, the extracts were centrifuged (Eppendorf, Germany) at 1000 × *g* for 15 min. The resulting supernatants were filtered to remove particulate matter. The filtered liquids were concentrated using a rotary evaporator under vacuum (Heidolph, Laborata 4003, Schwabach, Germany). The concentrated extracts were subsequently dried in an oven (Jeiotech, South Korea) at 45 °C until a constant weight was achieved. Dried extracts were stored in a refrigerator at 4 °C until further use (Rahnemoon et al., 2021).

Antioxidant activity of extracts

DPPH radical scavenging activity

The free radical scavenging capacities of methanolic and aqueous extracts of *S. hortensis* and *C. zeylanicum* were evaluated *in vitro* using DPPH assay following the methodology established by Blois (1958). Aliquots of 50 µL of each extract solution (1 mg/mL) were individually combined with 2 mL of methanol-based DPPH solution (24 µg/mL). The resulting mixture was mixed and incubated in the dark at room temperature for 60 min. Subsequent spectrophotometric analysis was performed at 517 nm to quantify radical scavenging activity (model Novaspec II; Pharmacia LKB, Uppsala, Sweden). DPPH radical scavenging activity was calculated using the following equation:

DPPH radical scavenging activity (%) = ($A_{blank} - A_{Sample}$)/ $A_{blank} \times 100$

Where A_{blank} is the absorbance of the blank (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Butylated hydroxytoluene (BHT) at a concentration of 1 mg/mL served as a positive control for comparative analysis. All tests were performed in triplicate.

Determination of ABTS radical scavenging activity

ABTS (7.00 mM) and potassium persulfate (2.45 mM) solutions were prepared. The solutions were combined and incubated for 16 h to generate ABTS + radical cations. The resulting solution was diluted with ethanol to achieve an absorbance of 0.70 ± 0.02 at 734 nm. Subsequently, 2 mL of this standardized solution was mixed with 200 µL of each extract (0.25 mg/mL concentration). Following a 1-minute incubation at room temperature, absorbance was measured at 734 nm using a spectrophotometer. (Novaspec II; Pharmacia LKB, Uppsala, Sweden). ABTS radical scavenging activity (%) was determined using the following equation:

ABTS radical scavenging activity (%) = ($A_{blank} - A_{sample}$)/ $A_{blank} \times 100$

where A_{blank} is the absorbance of blank (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. BHT at a 1 mg/mL is used as the reference compound (Valizadeh et al., 2023).

Tested microorganisms

The antifungal activity of methanolic and aqueous extracts of *S. hortensis* and *C. zeylanicum* was evaluated against four fungal strains, including *P. chrysogenum* ATCC 11709, and three *Candida* species including *C. albicans* ATCC 10231, *C. tropicalis* ATCC 750, and *C. dubliniensis* CD36.

Antifungal evaluation of extracts

MIC was determined using the broth microdilution method, as recommended by the Clinical Laboratory

Standards Institute (CLSI, M27-A3) (Pfaller et al., 2008), in 96-well flat-bottomed microtiter plates, using RPMI 1640, which had been buffered to pH 7.0 with 0.165 M MOPS. Furthermore, 100 µL of stock solution of each extract (20 mg/mL) was added to the first well containing 100 µL of medium and then serially diluted two-fold in the wells of 1–10 microdilution plates (10-0.019 mg/mL). The growth control well (well 11) was inoculated with 100 µL of sterile, antifungal-free medium and 100 µL of the inoculum suspension. The final well of the microdilution plates (well 12) was used as a sterility control (medium only). The yeast inoculum was adjusted to a concentration of 0.5×10^3 to 2.5×10^3 CFU/mL, and an aliquot of 100 µL was added to each well of the microdilution plate. The filamentous fungi inoculum was adjusted to a concentration of 0.5×10^4 to 1×10^4 CFU/mL, and an aliquot of 100 µL was added to each well of the microdilution plate. MICs were evaluated visually after a 24-hour incubation period at 35 °C for *Candida* species and at 30 °C for *Penicillium* over 48 h. The presence or absence of cellular growth was meticulously recorded, with growth observed in each experimental well compared with that in the control well.

MFC was defined as the lowest concentration of extracts that resulted in the absence of visible growth. This determination was conducted in triplicate by subculturing a 10 µL aliquot from all microdilution wells exhibiting no visible growth on SDA plates. The plates were subsequently incubated for 48 h at 35 °C to assess fungal growth. MFC was established as the lowest concentration of extracts that yielded no colonies in any of the MIC wells.

Statistical analysis

Data are presented as mean ± standard deviation of triplicate measurements using SPSS (IBM SPSS statistics V.27). Statistical analysis was performed using analysis of variance (ANOVA), followed by Duncan's test. $P < 0.05$ was considered to be significant.

Results and Discussion

Antioxidant activity of extracts

The present study investigated the antioxidant activities of aqueous and methanolic extracts of *C. zeylanicum* and *S. hortensis* using DPPH and ABTS assays. ABTS values were consistently higher than the DPPH results, likely because of broader sensitivity of ABTS to hydrophilic and lipophilic antioxidants (Floegel et al., 2011; Thaipong et al., 2006). Our findings indicate that in the DPPH assay, the methanolic extract of *C. zeylanicum* exhibited the highest antioxidant activity with a scavenging percentage of 89.16 ± 0.60%. The results were similar to the same concentration of BHT (91.50 ± 0.10). The aqueous extract of *C. zeylanicum* showed the lowest activity at 62.93 ± 3.85%. In the ABTS method, the methanolic extract of *C. zeylanicum* exhibited the highest antioxidant activity, with a scavenging percentage of 97.8 ± 0.1%. This result was closer to that of BHT at a concentration of 1 mg/mL (99.66 ± 0.15). Notably, the methanolic extract of *S. hortensis* showed significantly lower activity of 64.50 ± 1.73% (**Table 1**).

The antioxidant activities of *C. zeylanicum* and *S. hortensis* extracts can be attributed to their phenolic compounds (Fierascu et al., 2014; Davoudi et al., 2024). These results align with those of previous studies suggesting that methanolic extracts generally exhibit higher antioxidant activity than aqueous extracts because of the better solubility of bioactive compounds in methanol (Choi et al., 2007; Msaada et al., 2017). However, our findings for *S. hortensis* indicated a slight deviation from this trend, where the aqueous extract showed higher activity than the methanolic extract in both antioxidant tests.

Table 1. Antioxidant activity of aqueous and methanolic extracts of *Cinnamomum zeylanicum* (*C. zeylanicum*) and *Satureja hortensis* (*S. hortensis*) and BHT by DPPH (1 mg/mL of extracts) and ABTS (0.25 mg/mL of extracts) methods

Sample	Type of extract	DPPH	ABTS
C. zeylanicum	Aqueous	62.93 ± 3.85^{a}	82.9 ± 0.65^{b}
	Methanolic	89.16 ± 0.60^{c}	97.8 ± 0.10^{c}
S. hortensis	Aqueous	69.35 ± 4.15^{b}	82.6 ± 0.72^{b}
	Methanolic	66.26 ± 2.75^{ab}	64.5 ± 1.73^{a}
BHT (1mg/mL)	-	91.50 ± 0.10^{c}	99.66 ± 0.15^{d}

Values were presented as means ± standard deviation, Different small letters in each column (a-d) indicate statistically significant differences ($P < 0.05$). BHT: Butylated hydroxytoluene.

Table 2. Minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) of methanolic extract (ME) and aqueous extract (AE) of *C. zeylanicum* and *S. hortensis*.

Plant extracts (mg/mL)	**Microorganisms**							
	P. chrysogenum		***C. albicans***		***C. tropicalis***		***C. dubliniensis***	
	MIC	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
***C. zeylanicum* AE**	0.62 ± 0^{aB}	1.25 ± 0^{aA}	1.60 ± 0.7^{bB}	2.50 ± 0^{bB}	0.62 ± 0^{aB}	2.08 ± 0.7^{bB}	1.25 ± 0^{abC}	2.50 ± 0^{bAB}
***C. zeylanicum* MA**	0.31 ± 0^{bA}	0.62 ± 0^{aA}	0.15 ± 0^{aA}	1.25 ± 0^{bA}	0.31 ± 0^{bA}	0.62 ± 0^{aA}	0.31 ± 0^{bA}	1.25 ± 0^{bA}
***S. hortensis* AE**	5 ± 0^{cD}	6.60 ± 2.8^{bB}	2.50 ± 0^{bC}	2.50 ± 0^{aB}	0.31 ± 0^{aA}	2.5 ± 0^{aB}	2.5 ± 0^{bD}	3.30± 1.4^{aB}
***S. hortensis* ME**	1.25 ± 0^{cC}	2.50 ± 0^{bA}	0.31 ± 0^{aA}	1.25 ± 0^{aA}	0.31 ± 0^{aA}	2.5 ± 0^{bB}	0.62 ± 0^{bB}	1.25 ± 0^{aA}

Values were presented as means ± standard deviation. Different letters in each column (A-D) and row (a-c) indicate statistically significant differences ($P < 0.05$).

In a similar study (Mašković et al., 2017), various extracts of *S. hortensis* L. (summer savory) were prepared using conventional methods, such as maceration, Soxhlet extraction, and non-conventional techniques, including ultrasound, microwave, and subcritical water extraction. Notably, the subcritical water extract demonstrated the highest antioxidant activity, whereas Soxhlet extraction with a large amount of phenols yielded the lowest antioxidant potential. It is well established that antioxidant activity can be influenced by compounds with non-phenolic structures, which may also contribute to the observed effects. In another study, an ethanol: water (7:3) extract of *S. hortensis* demonstrated dose-dependent antioxidant activity in DPPH and ABTS assays, achieving > 90% inhibition at 400 µg/mL (Bahramikia, Yazdanparast, & Nosrati, 2008). The enhanced activity in hydroalcoholic extracts likely stems from the improved solubility of phenolic acids, such as rosmarinic acid, which correlates strongly with the antioxidant capacity of this species.

A comparative study (Singh et al., 2020) evaluated the antioxidant activity of *C. zeylanicum* extracts and

demonstrated that the methanolic extract exhibited the highest scavenging activity in the DPPH assay, achieving 94.97 ± 0.25% at 1000 µg/mL, which is similar to the results of the present study (89.16 ± 0.6%). Conversely, the ABTS assay revealed acetone extracts as the most effective, though the methanolic extract still showed notable activity (93.14 ± 0.40% at 1000 µg/mL), which was slightly lower than the 97.8 ± 0.1% recorded in this investigation (Singh et al., 2020).

Antifungal activity of *S. hortensis* extracts

The broth microdilution method was used to determine the antifungal activity of methanolic and aqueous extracts of *S. hortensis*. The results of MIC and MFC for *P. chrysogenum* and *Candida* species including *C. albicans*, *C. tropicalis*, and *C. dubliniensis* are shown in **Table 2**. The methanolic extract of *S. hortensis* exhibited significantly higher antifungal activity than the aqueous extract, with lower MIC and MFC values. This is evident from the lower MIC and MFC values against *P. chrysogenum*, in which the methanolic extract (MIC 1.25 mg/mL and MFC 2.5 mg/mL) was more potent than the aqueous extract (MIC 5 mg/mL and MFC 6.6 mg/mL). Furthermore, for *Candida* species, especially for *C. albicans*, methanolic extract MIC and MFC (MIC 0.31 mg/mL and MFC 1.25 mg/mL) were lower than aqueous extract.

A study conducted by Sahin et al. (2003) reported an MIC of 125–250 µg/mL against *C. albicans* for the methanolic extract of *S. hortensis*. However, no inhibition zone against *Penicillium* species was observed using the disk diffusion method when 300 µg/disc of the extract was tested. In comparison, the antifungal activity of the methanolic extract in this study was superior to that reported by Mohammed et al. (2019), who observed an MIC of 400 µg/mL against *C. albicans*. Conversely, the results showed lower activity than those reported by Adiguzel et al. (2007), where MIC values of 31.25 µg/mL and 62.5 µg/mL were recorded for *C. albicans* and *Penicillium* species, respectively, which were less than the MIC values observed in this study.

The enhanced efficacy of the methanolic extract of *S. hortensis* can be attributed to its ability to solubilize lipophilic compounds, such as thymol and carvacrol. Previous studies have shown that these phenolic compounds disrupt fungal cell membranes and inhibit ergosterol biosynthesis, which are vital for maintaining fungal cell integrity (Güllüce et al., 2003; Sahin et al., 2003; Sharifzadeh et al., 2016). Furthermore, our results are consistent with findings that showed methanolic extracts from other plant species exhibit superior antifungal activity compared to aqueous extracts owing higher concentrations of active compounds (Ahmad & Akram, 2019; Al-Zaben et al., 2023).

P. chrysogenum exhibited more resistance to both extracts and showed higher MIC and MFC values than *Candida* species. This aligns with molds' thicker, chitin-rich cell walls, which may impede extract penetration (Golparvar et al., 2018). Among *Candida* species, *C. albicans* was the most susceptible, with MICs as low as 0.31 mg/mL. The antifungal activity of methanolic and aqueous extracts of *S. hortensis* against *P. chrysogenum*, a common spoilage mold, highlights the use of this component as a natural preservative. In addition, low MICs against *Candida* species support the formulation for candidiasis treatment.

Antifungal activity of *C. zeylanicum* extracts

Cinnamon-derived substances exhibit potent antifungal properties and can function as fungistatic or fungicidal agents. These effects are attributed to the inhibitory actions of natural compounds in *Cinnamomum* that target fungal cellular structures and enzymatic systems. The mechanisms underlying these antifungal activities include cytoplasmic granulation, cytoplasmic membrane rupture, and inhibition or inactivation of intracellular and extracellular enzymes (Kačániová & Čmiková, 2025). These processes may occur independently or concurrently, ultimately suppressing mycelial germination (Cowan Marjorie, 1999). Furthermore, plant-derived lytic enzymes have been reported to degrade key structural polymers in fungal cell walls, such as 1,3-β-glucan, 1,6-β-glucan, and chitin, thereby compromising fungal integrity and viability (Brul & Coote, 1999).

The antifungal efficacy of *C. zeylanicum* varied significantly between the methanolic and aqueous extracts (**Table 2**), with solvent polarity playing a

critical role in potency. Methanolic extracts demonstrated superior antifungal activity; the enhanced antifungal activity observed with the methanolic extract of *C. zeylanicum* correlates with the role of methanol in efficiently extracting lipophilic bioactive compounds, such as cinnamaldehyde and other phenolic constituents, which are critical for the antimicrobial properties of *Cinnamomum* (Aneja et al., 2009; Correa-Royero et al., 2010; Mudaliar et al., 2013). The methanolic extract exhibited a MIC of 0.31 mg/mL and MFC of 0.62 mg/mL against *P. chrysogenum*, outperforming the aqueous extract (MIC 0.62 mg/mL and MFC 1.25 mg/mL). Similarly, in *Candida* species, methanolic extracts showed markedly lower MIC values than the aqueous extracts. A similar study demonstrated that the methanolic extract of *Cinnamomum* significantly inhibited the growth of *Aspergillus niger* and *C. albicans* compared to the aqueous extract. The methanolic extract showed MIC of more than 5 mg/ml against *C. albicans* and, hence, lesser antifungal activity than our results (MIC 0.15 mg/mL) (Badrinarayanan, Anand, Karpagam, Bai, & Ramasamy, 2014). In another study, the antifungal activity of *Cinnamomum* ethanolic extract was investigated against food-borne pathogens, which reported MIC of 2000 mg/mL against *Penicillium* species (Gupta et al., 2008). This MIC value was higher than that obtained in the present study for the aqueous and methanolic extracts of *cinnamon*. Among the *Candida* species, *C. albicans* demonstrated the highest susceptibility to the methanolic extract, whereas *C. tropicalis* and *C. dubliniensis* exhibited intermediate sensitivity (MIC 0.31 mg/mL). The reduced efficacy against the latter two species may reflect differential efflux pump activity or an enhanced biofilm-forming capacity.

Conclusion

In conclusion, this study highlights the potential of aqueous and methanolic extracts from *S. hortensis* and *C. zeylanicum* as potent antifungal agents against *P. chrysogenum* and *Candida* species. The extracts demonstrated robust antioxidant capabilities, as evidenced by their high scavenging percentages in the DPPH and ABTS assays. These findings underscore the importance of solvent choice in extracting bioactive compounds, and suggest that these plant extracts could serve as valuable natural alternatives for combating fungal infections and oxidative stress. Overall, this study contributes to the growing body of evidence supporting the therapeutic potential of plant-derived compounds and encourages further exploration of their applications in medicine and food preservation.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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