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PHENOLIC CONTENT, ANTIOXIDANT CAPACITY AND ANTIMICROBIAL ACTIVITY OF SELECTED NATURAL EXTRACTS: A COMPARATIVE ASSESSMENT

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ABSTRACT

Natural extracts represent promising sources of bioactive compounds for sustainable formulations. The aim of this study was to comparatively evaluate seven medicinal plant extracts and a 30 % propolis extract in terms of physicochemical characteristics, total phenolic content (TPC), DPPH radical-scavenging capacity, and antimicrobial activity. Propolis exhibited the highest TPC (1120 ± 45 mg GAE/L) and antioxidant activity (91.8 ± 1.2 %), while Thymus vulgaris showed the strongest performance among the botanical extracts. TPC was strongly correlated with DPPH activity ($r = 0.920$; $R^2 = 0.847$; $p = 0.0012$). Antimicrobial assays against Escherichia coli, Staphylococcus aureus, and Candida albicans revealed the lowest MIC values for propolis, followed by T. vulgaris, with S. aureus generally showing the greatest susceptibility. Pesticide residues were below the detection limit in all tested extracts. Based on their phenolic content, antioxidant capacity, and antimicrobial activity, propolis and T. vulgaris were identified as the most promising candidates for further evaluation as functional natural ingredients in sustainable formulations.

INTRODUCTION

Natural extracts obtained from medicinal and aromatic plants have attracted increasing scientific interest due to their complex phytochemical composition and potential antioxidant and antimicrobial functionality. Phenolic compounds, flavonoids, terpenoids, alkaloids, and other secondary metabolites contribute to these properties, although their abundance and biological effectiveness vary among botanical sources. *Rosmarinus officinalis* L. is characterized by a diverse phytochemical profile and documented biological properties (Andrade et al., 2018; Borges et al., 2019), including antioxidant and antimicrobial activity (Nieto et al., 2018).

Viola tricolor L. is another source of bioactive compounds with potential functional relevance. Phytochemical investigations of the genus Viola have demonstrated substantial diversity in secondary metabolites (Rosenhech et al., 2025; Wang et al., 2025), while studies on *V. tricolor* and related species have

reported phenolic compounds and antioxidant activity (Elida et al., 2016; Shahzadi et al., 2021). Its chemical diversity is further supported by the characterization of essential-oil constituents in Romanian plant material (Anca et al., 2009), and antimicrobial activity has also been reported (Witkowska-Banaszczak et al., 2005).

Several other medicinal species exhibit phytochemical profiles associated with biological activity. *Thymus vulgaris* L. contains constituents with recognized pharmacological properties (Prasanth Reddy et al., 2014), and its antimicrobial activity has been associated with the composition of its volatile fraction (Rota et al., 2008). *Salvia* species contain structurally diverse secondary metabolites (Wang, 2010), including diterpenes with considerable biosynthetic diversity (Cui et al., 2015). *Chelidonium majus* L. is characterized by a chemically distinctive profile and documented pharmacological properties (Gilca et al., 2010; Maji and Banerji, 2015), with particular attention given to its diverse alkaloid composition (Li et al., 2024). *Matricaria recutita* L. has demonstrated antimicrobial and antioxidant properties (Sharifi-Rad et al., 2018; Al-Dabbagh et al., 2019), supported by recent evidence concerning the biological properties of chamomile phytochemicals (Akram et al., 2024). Similarly, *Achillea millefolium* L. has a long history of ethnobotanical use and a diverse phytochemical profile (Chandler et al., 1982; Saeidnia et al., 2011; Ali et al., 2017). Propolis also represents a complex natural material with numerous biologically active constituents (Burdock, 1998), documented therapeutic potential (Sforcin, 2016), and well-established antibacterial properties (Przybyłek and Karpiński, 2019).

Despite extensive evidence concerning the phytochemistry and biological properties of individual medicinal plants and propolis, comparisons among independent studies remain difficult because extraction procedures, analytical protocols, extract concentrations, and biological test systems frequently differ. Evaluating chemically diverse natural extracts under a common experimental framework may therefore provide a more consistent assessment of the relationships among their phenolic content, antioxidant capacity, and antimicrobial performance. Accordingly, the present study aimed to comparatively characterize extracts of *Rosmarinus officinalis, Thymus vulgaris, Salvia officinalis, Viola tricolor, Chelidonium majus, Achillea millefolium*, and *Matricaria recutita*, together with a 30% propolis extract, by determining their physicochemical characteristics, total phenolic content, DPPH radical-scavenging capacity, and antimicrobial activity against *Escherichia coli, Staphylococcus aureus*, and *Candida albicans*. This comparative assessment was designed to identify differences in functional performance among the investigated natural extracts and to provide an experimental basis for selecting promising ingredients for subsequent development of natural and sustainable formulations.

MATERIAL AND METHODS

Eight commercially available natural extracts were investigated: *Rosmarinus officinalis* L., *Thymus vulgaris* L., *Salvia officinalis* L., *Viola tricolor* L., *Chelidonium majus* L., *Achillea millefolium* L., *Matricaria recutita* L., and a 30 % crude propolis extract. The plant extracts were standardized commercial hydroalcoholic preparations obtained from certified Romanian suppliers, whereas the 30% propolis preparation was an aqueous alcohol-free extract. An ethanol–water solvent control was included during antimicrobial testing to account for the hydroalcoholic extraction medium, as described below. The extracts were selected as natural raw materials with potential functional properties and were evaluated under the same laboratory

conditions to allow direct comparison of their physicochemical, antioxidant, and antimicrobial characteristics.

The physicochemical characterization included pH, electrical conductivity, dry matter content, and preliminary visual stability. The pH was measured after dilution of the extracts in distilled water (1:5 or 1:10, v/v), using a calibrated Mettler Toledo digital pH meter. Electrical conductivity was determined using the same pH/conductivity measurement system and expressed as μS cm^{-1}. Dry matter content was determined gravimetrically by controlled solvent evaporation followed by oven drying at 105 °C until constant mass. Preliminary stability was assessed during the first 72 h under ambient conditions by monitoring changes in color, odor, turbidity, and the formation of visible precipitates.

Total phenolic content (TPC) was determined using the Folin–Ciocalteu colorimetric method. Briefly, 0.1 mL of appropriately diluted extract was mixed with 0.5 mL of Folin–Ciocalteu reagent previously diluted 1:10 with distilled water. After 1 min, 1.5 mL of 7.5 % sodium carbonate solution was added, and the mixture was brought to a final volume of 10 mL with distilled water. Samples were incubated in the dark for 30 min at room temperature, and absorbance was measured at 765 nm using a UV–Vis spectrophotometer (Lambda 25, PerkinElmer). Gallic acid solutions ranging from 0 to 250 mg L^{-1} were used for calibration, and the results were expressed as mg gallic acid equivalents per liter of extract (mg GAE L^{-1}).

Antioxidant capacity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay. A 0.1 mM DPPH solution was prepared in methanol, and 1 mL of appropriately diluted extract was mixed with 2 mL of DPPH solution. The mixtures were gently homogenized and incubated for 30 min in the dark at room temperature. Absorbance was subsequently measured at 517 nm against a methanol blank. Radical-scavenging activity was calculated as DPPH inhibition (%) = $[(A_0 - A_s)/A_0] \times 100$, where A_0 represents the absorbance of the DPPH control and A_s the absorbance measured in the presence of the tested extract.

Antimicrobial activity was evaluated by a 96-well microdilution method through determination of the minimum inhibitory concentration (MIC). Three reference microorganisms were tested: *Escherichia coli* ATCC 25922 as a Gram-negative bacterium, *Staphylococcus aureus* ATCC 29213 as a Gram-positive bacterium, and *Candida albicans* ATCC 10231 as a fungal strain. Mueller–Hinton Broth was used for bacterial cultures and Sabouraud Dextrose Broth for *C. albicans*. Serial 1:2 dilutions of each extract were prepared over a concentration range of 100–0.1 % (v/v). Each well contained 100 μL of diluted extract and 100 μL of standardized microbial suspension (5×10^5 CFU mL^{-1}). Bacterial cultures were incubated at 37 °C for 24 h, whereas *C. albicans* was incubated at 30 °C for 48 h. Growth inhibition was initially evaluated by the absence of visible turbidity and subsequently confirmed by optical-density measurements at 600 nm using an ELx808 microplate reader (BioTek Instruments). Cell viability was additionally assessed using 0.015 % resazurin solution. Growth, reference antimicrobial, and solvent controls were included. The solvent control consisted of an ethanol–water solution (2:3, v/v) without plant extract. Ampicillin, vancomycin, and fluconazole were used as reference antimicrobial agents for *E. coli, S. aureus*, and *C. albicans*, respectively. MIC values were expressed as the lowest extract concentration (% v/v) producing complete inhibition of visible microbial growth.

Potential pesticide residues were evaluated by gas chromatography with electron-capture detection (GC–ECD). Approximately 2 g of each sample was

extracted with 10 mL of n-hexane by ultrasonication for 30 min, followed by centrifugation at 2500 rpm for 3 min. The resulting supernatant was concentrated under a nitrogen stream, reconstituted in 1 mL of n-hexane, and a 1 µL aliquot was analyzed using an Agilent Technologies 6890N GC–ECD system equipped with an HP-5 column. The injector and detector temperatures were maintained at 250 and 300 °C, respectively. The oven temperature was initially maintained at 80 °C for 5 min, increased at 2 °C min^{-1} to 275 °C, and maintained at the final temperature for 10 min. All physicochemical and spectrophotometric determinations were performed in triplicate, and quantitative results were expressed as mean ± standard deviation. The relationship between total phenolic content and DPPH radical-scavenging activity across the investigated extracts was evaluated using Pearson's correlation coefficient and simple linear regression, with statistical significance established at $p < 0.05$.

RESULTS AND DISCUSSIONS

The physicochemical characteristics of the investigated natural extracts showed noticeable variability among the analyzed materials. The pH values ranged within a relatively narrow acidic interval, from 4.60 ± 0.06 in *Salvia officinalis* to 5.30 ± 0.03 in *Viola tricolor*. Electrical conductivity varied more markedly, with the lowest value recorded for *Chelidonium majus* (970 ± 55 µS/cm) and the highest for the 30 % propolis extract (1560 ± 65 µS/cm). Dry matter content ranged from 4.8 ± 0.5 % in *Matricaria recutita* to 9.2 ± 0.3 % in *S. officinalis* among the botanical extracts, whereas the propolis extract showed a considerably higher value of 22.0 ± 0.1 %. During the 72-h preliminary stability assessment, no visible precipitation or marked deterioration was observed. Differences among extracts were mainly related to color, clarity and slight natural turbidity. The main physicochemical characteristics and preliminary visual stability of the investigated extracts are summarized in Table 1.

Table 1.
Physicochemical characteristics and preliminary visual stability of the investigated natural extracts.

Natural extract	pH (1:5 v/v)	Conductivity (µS/cm)	Dry matter (%)	Visual stability (72 h)
Rosmarinus officinalis	5.10 ± 0.05	1240 ± 50	7.5 ± 0.3	Clear, brown-yellow
Thymus vulgaris	4.80 ± 0.04	1100 ± 60	8.7 ± 0.4	Slightly turbid, brown-yellow
Salvia officinalis	4.60 ± 0.06	980 ± 45	9.2 ± 0.3	Clear, brown-green
Viola tricolor	5.30 ± 0.03	1020 ± 40	5.9 ± 0.4	Clear, lightly colored
Chelidonium majus	4.90 ± 0.05	970 ± 55	7.0 ± 0.3	Slightly opalescent
Achillea millefolium	4.70 ± 0.04	1050 ± 48	7.6 ± 0.2	Slightly turbid
Matricaria recutita	5.20 ± 0.05	1080 ± 42	4.8 ± 0.5	Clear, yellow
30% propolis extract	5.00 ± 0.02	1560 ± 65	22.0 ± 0.1	Brown, opaque

Total phenolic content differed considerably among the investigated extracts (Figure 1). The 30 % propolis extract showed the highest TPC, reaching 1120 ± 45 mg GAE/L. Among the botanical extracts, *Thymus vulgaris* exhibited the highest phenolic content (720 ± 30 mg GAE/L), followed by S. officinalis (680 ± 28 mg GAE/L) and *Rosmarinus officinalis* (560 ± 25 mg GAE/L). Intermediate values were obtained

for C. majus (510 ± 29 mg GAE/L) and *Achillea millefolium* (490 ± 31 mg GAE/L), while lower values were recorded for *V. tricolor* (470 ± 32 mg GAE/L) and *M. recutita* (450 ± 30 mg GAE/L). Thus, the phenolic content across all investigated materials ranged from 450 to 1120 mg GAE/L, revealing a distinct differentiation among the natural extracts.

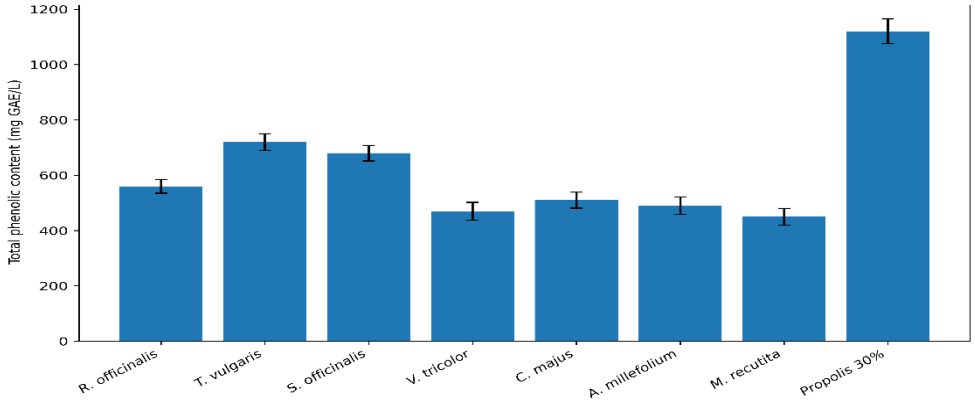


Figure 1. Total phenolic content (TPC) of the investigated natural extracts, expressed as mg gallic acid equivalents per liter (mg GAE/L). Values are presented as mean ± standard deviation (n = 3).

A similar pattern was observed for antioxidant capacity (Figure 2). The highest DPPH radical-scavenging activity was recorded for the 30 % propolis extract (91.8 ± 1.2 %), followed by *T. vulgaris* (85.6 ± 1.8 %) and *R. officinalis* (82.3 ± 2.1 %). S. officinalis also showed an inhibition value above 80% (80.2 ± 2.3%). Intermediate activities were observed for *C. majus* (78.4 ± 2.0%) and *A. millefolium* (76.5 ± 1.9 %), whereas *V. tricolor* and *M. recutita* exhibited the lowest values, at 74.1 ± 1.6 % and 72.3 ± 1.7 %, respectively. Nevertheless, all investigated extracts exhibited DPPH inhibition above 70 %.

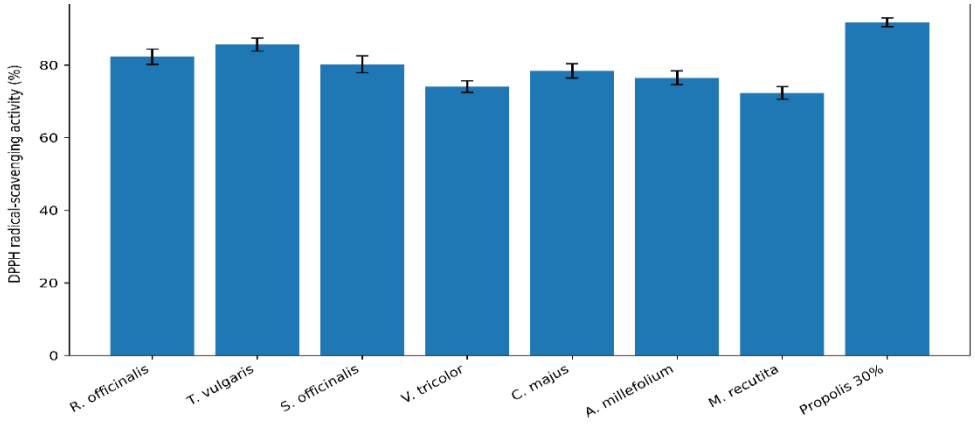


Figure 2. DPPH radical-scavenging activity of the investigated natural extracts. Values are expressed as percentage inhibition and presented as mean ± standard deviation (n = 3).

The relationship between total phenolic content and antioxidant activity is presented in Figure 3. Pearson correlation analysis indicated a strong positive association between TPC and DPPH radical-scavenging activity across the eight investigated extracts (r = 0.920, p = 0.0012). Simple linear regression resulted in an R^2 value of 0.847, indicating that extracts characterized by higher total phenolic contents generally exhibited higher DPPH inhibition under the experimental conditions. The 30 % propolis extract occupied the upper end of both variables, whereas *M. recutita* and *V. tricolor* were positioned toward the lower end of the observed ranges.

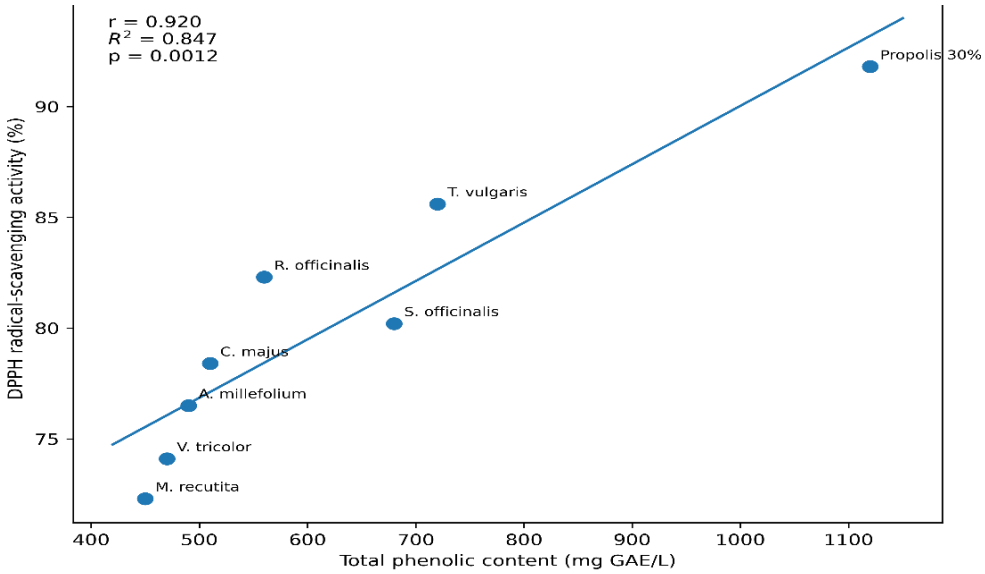


Figure 3. Relationship between total phenolic content (TPC) and DPPH radical-scavenging activity of the investigated natural extracts. The solid line represents the fitted simple linear regression (n = 8 extracts).

Antimicrobial activity also varied substantially among extracts and microorganisms (Figure 4). The 30 % propolis extract showed the lowest MIC ranges, with values of 1–2 % (v/v) against *E. coli*, 0.5–1 % against *S. aureus* and 2–4 % against *C. albicans*. *T. vulgaris* also exhibited comparatively low MIC values of 2–4 %, 1–2 % and 4–6 %, respectively. For C. majus, MIC values were 3.12 ± 0.6 % against E. coli, 2.50 ± 0.5 % against S. aureus and 6.25 ± 0.8 % against C. albicans. In contrast, V. tricolor showed MIC ranges of 10–12 %, 5–8 % and 12–16 %, respectively, while *M. recutita* presented corresponding ranges of 8–10%, 4–6% and 10–14 %. Across the investigated extracts, *S. aureus* generally showed the lowest MIC values, whereas *C. albicans* required higher extract concentrations for complete growth inhibition.

Pesticide residue analysis showed that pesticide residues were below the detection limit (<0.034 µg/kg) in all investigated extract samples. Therefore, no pesticide residues were detected in any of the analyzed extracts under the analytical conditions applied in this study.

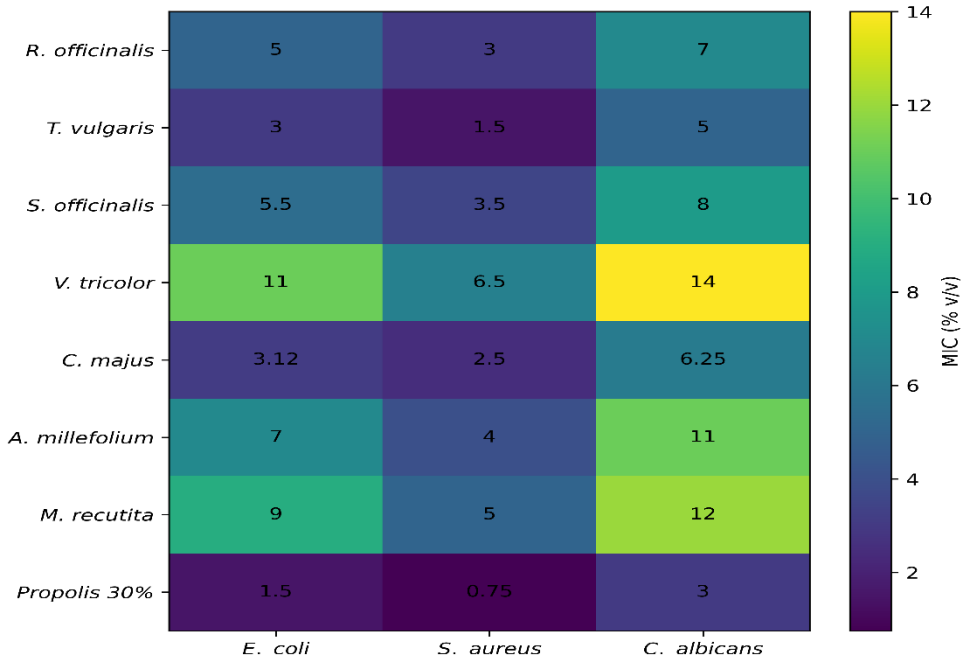


Figure 4. Heatmap of the minimum inhibitory concentration (MIC, % v/v) of the investigated natural extracts against *Escherichia coli, Staphylococcus aureus* and *Candida albicans*.

CONCLUSIONS

The comparative assessment demonstrated clear differences in the physicochemical, antioxidant, and antimicrobial properties of the eight investigated natural extracts. The 30 % propolis extract exhibited the strongest functional profile, combining the highest total phenolic content and DPPH radical-scavenging activity with the lowest MIC values against the tested microorganisms. Among the botanical extracts, *Thymus vulgaris* showed particularly favorable performance, with high phenolic content and antioxidant capacity together with comparatively strong antimicrobial activity. *Rosmarinus officinalis* and *Salvia officinalis* also displayed high antioxidant activity, whereas *Viola tricolor* and *Matricaria recutita* showed lower antioxidant and antimicrobial performance under the experimental conditions. A strong positive relationship was observed between total phenolic content and DPPH radical-scavenging activity ($r = 0.920$; $R^2 = 0.847$; $p = 0.0012$), indicating that phenolic content was closely associated with antioxidant performance across the investigated extracts. Antimicrobial susceptibility was microorganism-dependent, with *Staphylococcus aureus* generally showing greater sensitivity and *Candida albicans* requiring higher extract concentrations for growth inhibition. The results identify propolis and *T. vulgaris* as the most promising candidates for further investigation and support the use of comparative phytochemical and biological screening as a basis for selecting natural ingredients for sustainable formulations. Further studies should evaluate their performance after incorporation into final formulations, including long-term stability, optimal concentrations, and functional efficacy under practical conditions.

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