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Short communication

Determination of total phenolic and flavonoid content in selected antioxidant herbal drugs

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Introduction

Medicinal plants represent an important source of biologically active compounds, including phenolic acids, flavonoids, tannins, anthocyanins and other secondary metabolites with significant antioxidant properties. Due to their ability to neutralize reactive oxygen species and prevent oxidative damage, plant-derived antioxidants have attracted considerable attention in pharmaceutical, nutraceutical and cosmetic research (Dai and Mumper, 2010). *Achillea millefolium*, *Verbena officinalis*, *Camellia sinensis*, *Hibiscus sabdariffa*, *Rosa canina* and *Trifolium pratense* have been extensively investigated because of their diverse phytochemical composition and traditional medicinal use. These species contain various classes of phenolic compounds, flavonoids, catechins, anthocyanins and isoflavones that contribute to their biological activities (Dai and Mumper, 2010).

The aim of this study was to quantify the total phenolic and flavonoid contents of selected medicinal plant extracts and their combinations, and to investigate their potential as natural sources of antioxidant phytochemicals.

Materials and methods

Materials

Glycolic extracts of *Achillea millefolium*, *Verbena officinalis*, *Trifolium pratense*, and *Rosa canina* were obtained from MPG LAB (Slovenia) while dry extracts of *Hibiscus sabdariffa* (10%) and *Camellia sinensis* (90%) were supplied by Nexira (France). Two plant-extract combinations were prepared and mixed in a 1:1:1 ratio. The Red Combination consisted of *Hibiscus sabdariffa*, *Rosa canina* and *Trifolium pratense* extracts, while the Green Combination consisted of *Achillea millefolium*, *Verbena officinalis*,and *Camellia sinensis* extracts.

Total phenolic content (TPC)

Total phenolic content (TPC) was determined spectrophotometrically using the Folin–Ciocalteu method and expressed as gallic acid equivalents (Singleton et al., 1999).

Total flavonoid content (TFC)

Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric assay (Chang et al., 2002).

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All analyses were performed in triplicate and the obtained results were used for comparative assessment of the phenolic and flavonoid content of the investigated medicinal plant extracts.

Results and discussion

The total phenolic content (TPC) varied among the investigated plant extracts, ranging from 4.68 to 7.97 mg/mL. The highest TPC was determined in *Camelia sinensis* (7.97 mg/mL), followed by *Achillea millefolium* (7.35 mg/mL), *Verbena officinalis* (7.03 mg/mL), *Rosa canina* (6.73 mg/mL), and *Trifolium pratense* (6.03 mg/mL), while *Hibiscus sabdariffa* exhibited the lowest value (4.68 mg/mL). The prepared Red Combination showed a TPC of 6.95 mg/mL, whereas the Green Combination exhibited a slightly higher value of 7.21 mg/mL.

The total flavonoid content (TFC) demonstrated greater variability among the extracts, with values ranging from 4.69 to 15.12 mg/mL. The highest flavonoid content was observed in *Verbena officinalis* (15.12 mg/mL), followed by *Camelia sinensis* (14.31 mg/mL), *Trifolium pratense* (9.03 mg/mL), *Achillea millefolium* (8.28 mg/mL), *Rosa canina* (5.38 mg/mL) and *Hibiscus sabdariffa* (4.69 mg/mL). The Green Combination exhibited a substantially higher TFC (13.48 mg/mL) compared to the Red Combination (8.40 mg/mL), reflecting the contribution of the flavonoid-rich extracts included in its composition.

Phenolic and flavonoids are among the most important plant secondary metabolites responsible for the antioxidant activity of medicinal plants. Their ability to scavenge free radicals, chelate metal ions, and modulate oxidative processes contributes significantly to the biological effects of plant-derived extracts (Dai and Mumper, 2010). The relatively high TPC and TFC values observed for the Green Combination suggest a greater abundance of bioactive constituents, which may enhance its antioxidant potential. In particular, the elevated flavonoid content can be attributed to the presence of *Verbena officinalis* and *Camelia sinensis*, both recognized as rich sources of flavonoid compounds (Liga et al., 2023). Although the Red Combination exhibited lower TFC values, its considerable phenolic content indicates that it also represents a valuable source of antioxidant phytochemicals. The observed phenolic and flavonoid profiles suggest that both formulations may provide protection against oxidative stress, a process closely associated with tissue damage and impaired wound healing (Shi et al., 2022).

Conclusion

The investigated plant extracts exhibited considerable levels of phenolic compounds and flavonoids, with *Camelia sinensis* and *Verbena officinalis* showing the highest contents. Among the prepared formulations, the Green Combination demonstrated slightly higher total phenolic content and substantially higher total flavonoid content than the Red one. These findings indicate that both formulations represent promising sources of bioactive phytochemicals, with the Green Combination showing particular potential for antioxidant-related applications due to its enriched flavonoid profile.

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