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ABSTRACT BOOK

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Non-aqueous DPPH spectrophotometric assay for evaluating lipophilic compounds with tocopherol acetate calibration

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Background: For assessing antioxidant activity of hydrophilic and lipophilic compounds, hydrophilic standards (Trolox, ascorbic acid, gallic acid, rutin, etc.) are most commonly used in spectrophotometric studies. However, for lipophilic substances, it is more appropriate to perform the analysis in non-aqueous conditions and compare their activity with a lipophilic standard. α-Tocopherol serves as such a standard, demonstrating activity comparable to Trolox (1). Therefore, a DPPH-based method in a non-aqueous medium was developed using α-tocopherol as a standard and applied to chamomile essential oil.

Aim(s): To develop and apply a non-aqueous DPPH spectrophotometric method for the assessment of antiradical activity of lipophilic compounds using tocopherol acetate as a lipophilic reference standard, and to express the activity of chamomile essential oil in tocopherol equivalents.

Methods: Tocopherol acetate was dissolved in methanol and diluted (1000–62.5 μg/mL). DPPH (0.003 g/100 mL) in acetonitrile was mixed with 50 μL of tocopherol solution in 5.0 mL and incubated 20 min. Absorbance at 517 nm was measured in triplicate; calibration curve: $y = 0.0005x + 0.0141$ ($R^2 = 0.9994$). Chamomile oil (400 μL in 5 mL methanol) was analyzed similarly; absorbance converted to tocopherol equivalents.

Results: A linear calibration curve for tocopherol acetate in 62.5–1000 μg/mL was obtained, demonstrating excellent linearity ($R^2 = 0.9994$). The antiradical activity of chamomile essential oil under non-aqueous conditions corresponded to 67.8 μg/mL tocopherol acetate, equivalent to ~3.39 μg per reaction mixture (50 μL added). The analyzed oil in the mixture was ~3.70 μL (50 μL of 7.41% v/v solution).

Conclusion: A non-aqueous DPPH method suitable for lipophilic compounds was developed using tocopherol acetate. This enables expressing essential oil activity in tocopherol equivalents, more appropriate than hydrophilic standards in non-aqueous systems. Further studies will integrate HPTLC and GC to determine contributions of individual components.

References:

1. Castro IA, Rogero MM, Junqueira RM, Carrapeiro MM. Free radical scavenger and antioxidant capacity correlation of alpha-tocopherol and Trolox measured by three in vitro methodologies. J Nutr Biochem. 2006;17(12):801–7. doi: 10.1080/09637480600656199. PMID: 16849116.