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Rapid HPTLC-based method for quality control: simultaneous chemical analysis and antioxidant activity determination in herbal, nutraceutical and functional foods

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Abstract

Antioxidants belong to one of the most important group of compounds presented in herbs, nutraceuticals and functional food, which prevent oxidative stress avoiding cell damage. Hence, antioxidants have a high demanding and also their natural sources. A new procedure has been used to separate and quantify the free radical-scavenging activity of individual compounds from 44 samples of *Calendula officinalis*, 18 samples of *Thymus vulgaris* and 12 samples of *Rosmarinus officinalis,* based on the combination of HPTLC with a diode array detector (DAD) and postchromatographic DPPH$^{\bullet}$ radical derivatization.

Keywords: HPTLC; Antioxidants; Calendula officinalis; Thymus vulgaris; Rosmarinus officinalis

1. Introduction

Plant secondary metabolites, such as essential oils and flavonoids, have been widely studied for their antimicrobial, insecticidal, antifungal, antibacterial and cytotoxic activities [1]. They are intensely screened and applied in the fields of pharmacology, medical and clinical microbiology, phytopathology and food preservation [2]. Nowadays, there is an increasing interest in natural antioxidants; particularly for phenols intended to prevent not only the presumed deleterious effects of free radicals in the human body, but also the deterioration of fats and other constituents of foods. The antioxidant property of

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essential oils also has been verified in vitro by physical–chemical methods to promote their use as natural food additives [3].

Thymus vulgaris (*T. vulgaris*) and *Rosmarinus officinalis* (*R. officinalis*) as well as many other aromatic plants biosynthesize high amount of volatile compound referred as the essential oil. In *T. vulgaris,* among the major compounds available in the oil, thymol and carvacrol were reported that possess the highest antioxidant activity [4]. In addition, these compounds exhibit other biological activities. Thymol is an antiseptic, while carvacrol possesses antifungal properties [5]. Non-volatile antioxidants such as flavonoids and vitamin E were also found in the extracts of *T. vulgaris* [6]. Therefore, the whole plant and its essential oils and non-volatile compounds of thyme can be used as natural preservative and antioxidants ingredients in the food industries [7]. *Rosmarinus officinalis* (*R. officinalis*) is probably one of the most exploited species for its essential oil, phenols and biological properties as preservative in foods [8]. The essential oil content monoterpenes such as borneol, camphor, terpene-4-ol, linalool, α-terpeneol, monoterpene hydrocarbons such as α-pinene, camphene, β-pinene, myrcene, α-phellanderene, 1,8-cineole, trans β-ocimene, γ-terpenene, and cissabinene hydrate [8]. *Calendula officinalis* (*C. officinalis*) commonly known as marigold is particularly identified for having a bright yellow and orange flower. The flower is normally used as food additive for both color and flavor to foods [9]. This plant is also widely used in traditional and homeopathic medicine as infusions and ointments prepared with its petals. It presents several therapeutic activities, such as anti-inflammatory, antitumorogenic and cicatrizing ones [10]. The most important compounds are triterpenoids, flavonoids, essential oils and sesquiterpenes. Flavonoids and phenolic compounds in *C. officinalis*, which exhibit antioxidant activity, have high interest in food industries as well as in phytotherapy.

Although, antioxidants have a high demanding and also their sources, there is a great problem for assurance raw material quality due the variability of metabolite content. This work uses HPTLC to compare quality in terms of metabolite content and antioxidant capacity. 1, 1-Diphenyl-2-picrylhydrazyl radical ($DPPH^{\bullet}$) is a popular free radical used in assessing the antioxidant activity. Having chromatographic separation and antiradical activity determination allows evaluate the antioxidant contribution different component in the extract.

A new procedure has been applied to separate and quantify the free radical-scavenging activity of individual compounds from *C. officinalis, T. vulgaris* and *R. officinalis* extract based on the combination of HPTLC with a diode array detector (DAD) and postchromatographic $DPPH^{\bullet}$ radical derivatization. The main purpose of this work was to determine and compare the differences in metabolite content in 44 samples of *C. officinalis*, 18 samples of *T. vulgaris* and 12 samples of *R. officinalis* from different ecotypes and geographical origins. Owing to the metabolite expression is correlated with the biological activity.

2. Materials and Methods

2.1. Plant material

C. officinalis, *T. vulgaris* and *R. officinalis* were botanically identified and voucher specimens were deposited at the Herbarium of Universidad de Antioquia HUA, (*T. vulgaris*: Alzate 3438, *C. officinalis*: Alzate 3444 and *R. officinalis*: Alzate 3442). From *T. vulgaris*, 3 different origins were evaluated: T1, T2 and T3 and they were harvested in 6 different agro ecological places in Antioquia state (Colombia) Rio Negro (RN), La Ceja (LC), Marinilla (M), Peñol (P), Guarne (G) and Santuario (S). For *R. officinalis* only 2 different origins were evaluated R1 and R2 and they were cultivated in the 6 places mentioned for *R. officinalis*. For *C. officinalis* 9 different origins were used: CI6A, C11C, CI8K, C4, C9, C2, C2I, C9A, all of them cultivated in one location: Rio Negro. The samples were dried at 45ºC in an air-forced dryer (Dies 2009, Colombia), ground in mill-excelsior (IKA A11 Basic). The powder was stored in closed vessel for further use.

2.2. Chemicals

Ethyl acetate, toluene, and formic acid, ethyl formiate were of analytical grade and purchased from Merck (Darmstadt, Germany), methanol was of HPLC grade from Merck, DPPH$^{\bullet}$ was from Sigma (St. Louis, MO, USA). Water was purified by a Milli-Q system. The used reference compounds rutin, rosmarinic acid and thymol were from Chromadex.

2.3. HPTLC Analysis

Aluminium sheets Kieselgel 60 from Merck (Darmstadt, Germany) were used. Samples were applied with a 100 uL sample syringe (Hamilton, Bonaduz, Switzerland) using a Linomat V system (Camag, Switzerland). Samples of 5 uL were applied as 5 mm bands. Plates were developed in a vertical glass chamber (Camag, Switzerland) for 5 min using toluene/ ethyl acetate (97:3 by volume) as a mobile phase for *T. vulgaris*, toluene/ethyl formiate/formic acid for *R. officinalis* and ethyl acetate/formic acid/glacial acetic acid/water (100:11:11:26 by volume) for *C. officinalis*. After development, the plates were dried and the components were visualized by UV irradiation at 280 nm for tymol, 250 nm for rosmarinic acid and 360 nm for rutin. Then the plates were dipped into a 0.05% DPPH$^{\bullet}$ solution and monitoring at 518 nm. Antiradical activity of each component of the extract was estimated on the intensity of disappearance of violet/purple background of plate and was quantified by densitometric scanning at 518 nm as negative peak (Camag TLC scanner 3 under software control of WinCats). Each determination was carried out in duplicate. In order to calibrate the method, stock solutions of the reference compound were prepared in methanol at different concentration levels, these solutions were analyzed by HPTLC exactly as described above and calibration curves were prepared by plotting the negative peak area versus concentration. The antioxidant activity was expressed as ng of rutin equivalent/mg of sample.

3. Results and Discussion

Chromatographic separation, in situ radical-scavenging activities and quantitative analysis of secondary metabolites of *T. vulgaris*, *R. officinalis* and *C. officinalis* aerial parts extract were carried out by on-line HPTLC-DAD and HPTLC-DPPH$^{\bullet}$ methods. Thanks to the simultaneous separation and scanner determination, it was possible to test extracts without extensively preparation.

The compounds thymol, rosmarinic acid and rutin from *T. vulgaris*, *R. officinalis* and *C. officinalis* extracts, respectively, were identified by comparisons of their Rf values and UV spectra to standards analyzed under identical analytical conditions, while the quantitative data were calculated from their calibration curves. For *T. vulgaris* 3 different origins were evaluated: T1, T2 and T3 and they were cultivated in 6 different agroecological places in Antioquia state (Colombia): Rio Negro (RN), La Ceja (LC), Marinilla (M), Peñol (P), Guarne (G) and Santuario (S). For *R. officinalis* only 2 different origins were evaluated R1 and R2 and they were cultivated in the 6 places mentioned for *R. officinalis*. For *C. officinalis* 44 different samples were tested (Data not shown) but only 9 were selected based on the metabolite content CI6A, C11C, CI8K, C4, C9, C2, C2I, C9A, all harvested in Rio negro town. The antioxidant activity is expressed as ng of rutin-equivalent/mg, which represents the amount in ng of any compound or group of compounds, present in 1mg of dry sample, with the same antioxidant activity of rutin. The calibration curves of negative peaks of rutin were obtained from a calibration solutions developed in the HPTLC and postchromatographic DPPH$^{\bullet}$ radical derivatization. A linear correlation was found between the amount of analyte and the negative peak area (r^2: 0.9735, n=4). The method was applied to measure the free radical scavenging activity of thymol in *T. vulgaris*, rosmarinic acid in *R. officinalis* and chlorogenic acid, hyperosid and rutin in *C. officinalis*.

The antioxidant activity amongst the 18 samples of *T.vulgaris* was significantly different (p-value< 0.0001). For *T. vulgaris*, the best material for expressing the metabolite responsible of the antioxidant

activity, thymol, is T3, cultivated in Santuario (S) (T1S: $2.37x10^4 \pm 4.42x10^2$; T2S: $2.00x10^4 \pm 1.06x10^3$, T3S: $2.83x10^4 \pm 2.70x10^3$ ng rutin-eqv/mg) and in Guarne (G) (T1G: $1.38x10^4 \pm 3.58x10^2$; T2G: $1.94x10^4 \pm 1.65x10^2$; T3G: $2.47x10^4 \pm 4.83x10^1$ng rutin-eqv/mg ± SD) (Fig. 1a). *R. officinalis* had a different behavior, in this case the best agro ecological places are Rio Negro (RN) (R1RN: $8.03x10^3 \pm 1.09x10^2$; R2RN: $8.84x10^3 \pm 3.46x10^2$) and La Ceja (LC) (R1LC: $7.38x10^3 \pm 6.87x10^1$, R2LC: $1.18x10^4 \pm 1.95x10^3$). The antioxidant activity in the 12 samples tested was significantly different (p-value< 0.0001) (Fig. 1b). For *C. officinalis* the content of ng rutin-eqv/mg is expressed for a group of three compounds (chlorogenic acid, hyperosid and rutin), the 9 materials evaluated, are significantly different in terms of their antioxidant activities (p-value <0.0001) (Fig. 1c).

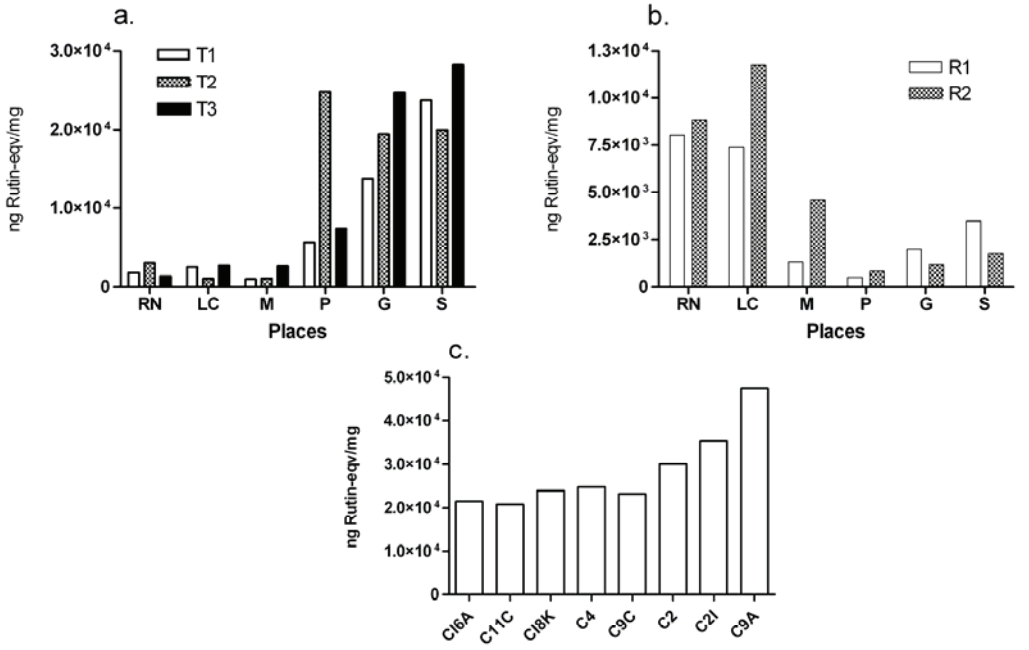

Fig. 1. (a) ng of rutin-equivalent/mg of sample for *T. vulgaris* from Rio Negro (RN), La Ceja (LC), Marinilla (M), El Peñol (P), Guarne (G) and Santuario (S); (b)ng of Rutin-equivalent/mg of sample for *R. officinalis* from Rio Negro (RN), La Ceja (LC), Marinilla (M), El Peñol (P), Guarne (G) and Santuario (S); (c) ng of Rutin-equivalent/mg for *C. officinalis* from Rio Negro

4. Conclusion

It has been well reported that not only that the biomass yield but also the metabolite content in herbs, depend on genetics and on the agro ecological conditions, due to the great effect of the weather, rains, altitude among other factors [8]. According to the antioxidant activity of the materials evaluated, it was observed that the effect of the ambient on the antioxidant profile for the species tested is extremely significant. The procedure could be used for rapid analysis in pharmaceutical and food industries where standardization of raw material plays a key role on quality and efficacy of final products. Plant extracts have complex structures, and isolation and identification of active compounds involve difficult, long, and expensive processes. The post chromatographic derivatization of plates in the HPTLC-DPPH$^{\bullet}$ method used in this study can be successfully used for the qualitative and quantitative analysis of free radical

scavengers in complex mixtures. This study has established that some of the detected compounds of the Calendula officinalis, Thymus vulgaris and Rosmarinus officinalis extracts were able of scavenging $DPPH^{\bullet}$ radicals. Therefore postchromatographic derivatization techniques can be used as a cheap, fast, and efficient alternative. This work pretends to demonstrate the great importance of the use of new technologies in quality control. We applied an in situ methodology to evaluate the antioxidant activity of compounds present in the plants, which could be a roughest application based not only in chemical composition but also in antioxidant activity.

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References

[1] Faleiro L, Miguel GM, Guerrero CAC, Brito JMC. Antimicrobial activity of essential oils of *Rosmarinus officinalis* L., *Thymus mastichina* (L) L. ssp. *mastichina* and *Thymus albicans*. II Wocmap congress on medicinal and aromatic plants, part 2: pharmacognosy, pharmacology, phytomedicine, toxicology; Mendoza. Argentina,1999.

[2] Daferera DJ, Ziogas BN, Polissiou MG. GC–MS Analysis of essential oils from some Greek aromatic plants and their fungitoxicity on Penicillium digitatum. *J Agric Food Chem* 2000;**48**:2576-2581.

[3] Ruberto G, Baratta MT. Antioxidant activity of selected essential oil components in two lipid model systems. *Food chem* 2000;**69**:167-174.

[4] Sarikurkcu C, Sabih Ozer M, Eskici M, Tepe B, Can S, Mete E. Essential oil composition and antioxidant activity of *Thymus longicaulis C. Presl subsp. longicaulis var. longicaulis*. *Food Chem Toxicol* 2010;**48**(7):1801-1805.

[5] Menphini A, Pagiotti R, Capuccella M. Antifungal activity of carvacrol chemotypes of winter savory harvested in Italy. *Rivita Italiana EPPOS* 1993;**4**:566-571.

[6] Dapkevicius A, van Beek TA, Lelyveld GP, Veldhuizen v, E dGA, Linssen JPH. Isolation and structure elucidation of radical scavengers from *Thymus vulgaris* leaves. *J. Nat Prod* 2002; **65**:892-896.

[7] Nguefack J, Dongmo JBL, Dakole CD, Leth V, Vismer HF, Torp J, et al. Food preservative potential of essential oils and fractions from *Cymbopogon citratus*, *Ocimum gratissimum* and *Thymus vulgaris* against mycotoxigenic fungi. *Int J Food Microbiol* 2009;**131**(2-3):151-156.

[8] Zaouali Y, Bouzaine T, Boussaid M. Essential oils composition in two *Rosmarinus officinalis* L. varieties and incidence for antimicrobial and antioxidant activities. *Food Chem Toxicol* 2010;**48**(11):3144-3152.

[9] Danielski L, Campos LMAS, Bresciani LFV, Hense H, Yunes RA, Ferreira SRS. Marigold (*Calendula officinalis* L.) oleoresin: Solubility in SC-CO_2 and composition profile. *Chem Eng Process* 2007;**46**(2):99-106.

[10] M. Hamburger S, Adler DB, Forg A, Weinreich B. Preparative purification of the major anti-inflammatory triterpenoid esters from marigold (*Calendula officinalis*). *Fitoter* 2003;**74**:328-338.

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