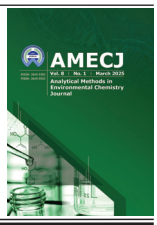
Anal. Methods Environ. Chem. J. 8 (1) (2025) 78-99

AMECJ

Research Article, Issue 1
Analytical Methods in Environmental Chemistry Journal
Journal home page: www.amecj.com/ir



Determination and investigation of phenolic compounds in Citrus pulps and zests by Ultra-high performance liquid chromatography

Amal Sammama [a,b,*], Hasnaa Sammama[c,d], Rachid Bengueddour [a]

[a]*Natural Resources and Sustainable Development Lab, Department of Biology, Faculty of Science, Ibn Tofail University, Kenitra, Morocco*

[b]*Center of Analysis and Characterization, University Cadi Ayyad, Marrakech, Morocco*

[c]*Laboratory of Phyto-Bacteriology, Plant Protection Research Unit, National Institute of Agricultural Research, Marrakesh, Morocco*

[d]*Laboratory of Agro-Biotechnology and Bioengineering, Department of Biology, Faculty of Sciences and Technologies-Gueliz, Cadi Ayyad University, Marrakesh, Morocco*

ARTICLE INFO:

Received 21 Nov 2024

Revised form 27 Jan 2025

Accepted 18 Feb 2025

Available online 30 March 2025

Keywords:
Antioxidant activities;
Analysis;
Citrus;
Phenolic composition;
Ultra-high-performance-liquid-chromatographic-diode array;
2.2-diphenyl-1-picrylhydrazyl

ABSTRACT

This study aimed to evaluate in vitro the influence of extraction methods (maceration and soxhlet) and solvents (hexane, dichloromethane, ethyl acetate and methanol) on the phenolic composition and antioxidant activity of tree citrus species: *C. limon, C. limetta and C. aurantifolia*. The results showed that the soxhlet remains the best extraction method for quantifying polyphenols, flavonoids and condensed tannins. Polar solvents (ethyl acetate and methanol) make it possible to obtain the best extraction yields and high polyphenols and flavonoids. *Citrus aurantifolia* shows the highest contents for epicarps and pulps, respectively in polyphenols (326.73 mg and 443.57 mg Gallic acid equivalent (EAG) per gram of fresh weight; FW) and in flavonoids (133.00 mg and 138.62 mg Equivalent (EQ) per gram of FW). The evaluation of the antioxidant activity using the 2.2-diphenyl-1-picrylhydrazyl (DPPH) for the different extracts showed that methanol and ethyl acetate extracts by soxhlet possess the best antioxidant activities. A strong antiradical power was noted for the epicarps and the pulps of *C. aurantifolia* fruit, respectively of 5.13 and 4.89 µg mL^{-1}. 15 phenolic compounds were tentatively identified by an ultra-high-performance-liquid-chromatographic-diode array (UHPLC-DAD) in the polar *Citrus* pulp and epicarp extracts. In fact, the identification of three flavonols (rutin, kaempherol and quercetin), two flavanones (hesperidin and naringin), a flavone (3,4,5,7-tetrahydroxyflavone)and a phenolic acid (pyrogallic acid) were the major compounds identified in polar citrus pulp and epicarp extracts.

1. Introduction

According to current nutritional theories, one secret to good health is to absorb more antioxidants, which promote the proper aging of the body's various organs. This is one of the major reasons why a Mediterranean diet rich in fresh fruit and vegetables is recommended [1].

The ingestion of secondary metabolites via fruit and vegetables could enable our bodies to strengthen their defenses against the oxidation processes that threaten our cells on a daily basis. However, the mechanisms involved probably go far beyond the direct reduction of reactive oxygen species by secondary metabolites such as phenolic compounds [2]. *Citrus* (*Citrus L. de Rutaceae*) is one of the world's major fruit crops. It is widely cultivated in tropical and subtropical

*Corresponding Author: Amal Sammama
Email: sammama.amal@gmail.com
https://doi.org/10.24200/amecj.v8.i01.1006

regions of the world and in many other areas. *Citrus* cultivation is the world's largest fruit production sector, with more than 161.8 million tons annually in 2021 [3]. *Citrus* is a highly valuable matrix due to its wealth of functional ingredients (essential oils, fibers, carotenoids, vitamin C, phenolic compounds) with a wide range of applications in the food, cosmetics and nutraceutical industries, as well as in the production of biofuels and biodegradable materials [4]. However, various epidemiological studies have suggested that citrus fruits positively affect a range of degenerative disorders [5]. *Citrus* consumption has expanded dramatically in recent years due to its beneficial effects on human health. *Citrus* peels represent a major source of phenolic compounds, mainly flavonoids and phenolic acids. *Citrus* flavonoids include glycosylated flavanones and polymethoxylated flavones [6]. *Citrus* peel flavonoids are characterized by their antioxidant, therapeutic, antiviral, antifungal and antibacterial activity [7]. Indeed, recent research has focused on by-products that extract natural antioxidants to replace synthetic antioxidants [8]. In addition, most industries have focused on fruit and vegetable by-products to transform them into exploitable products [9]. Indeed, our study evaluate firstly the effect of extraction methods (maceration and soxhlet) and the different solvents (hexane, dichloromethane, ethyl acetate and methanol) on the quantification of total phenols, flavonoids and condensed tannins contained in *Citrus Limon Burm, Citrus Limetta Risso and Citrus Aurantiifolia (Christm.) Swingle*. Secondly, evaluating the effect of the extraction methods and the different solvents on the antioxidant activity of the studied extracts. Thus, the various phenolic compounds of polar citrus extracts are characterized by UHPLC-DAD.

2. Material and Methods

2.1. Plant material

At the maturity stage, the fruits of C. limetta, C. limon, and C. Aurantiifolia were randomly harvested from all the trees. The fruits were washed, the pulp and epicarp were manually separated and seeded. 25.0 g of fresh pulp or epicarp was extracted by maceration (250 ml) and soxhlet (300 mL) in increasing solvent polarity for 24h at room temperature and in the dark. The extracts were evaporated using a rotavapor, then taken up with various volumes of methanol and stored at -4°C.

2.2. Reagents

All thirty-three standards used for analyses were obtained from Sigma-Aldrich (Berin, Germany): blend 1: pyrogallic acid (CAS Number: 87-66-1), vannilic acid (CAS Number: 121-34-6), caffeic acid (CAS Number: 331-39-5), ferulic acid (CAS number: 1135-24-6), hesperidin (CAS Number: 520-26-3) and salicylic acid (CAS Number: 69-72-7). The blend two including: Gallic acid (CAS Number: 149-91-7), Catechin (CAS Number: 7295-85-4), chlorogenic acid (CAS Number: 327-97-9), epicathechin (CAS Number: 490-46-0), vanillin (CAS Number: 121-33-5), p-coumaric acid (CAS Number: 501-98-4), sinapic acid (CAS Number: 530-59-6), naringin (CAS Number: 10236-47-2), rutin (CAS number: 153-18-4), quercetin (CAS number: 117-39-5) and kaempferol (CAS number: 520-18-3). Also, the blend three including: catechol (CAS number: 120-80-9), hydroxybezoic acid (CAS Number: 99-96-7), syringic acid (CAS Number: 530-57-4), 3,4 dimethoxybezoic acid (CAS Number: 93-07-2), 2-hydroxynaphthoic acid (CAS Number: 2283-08-1), rosmarinic acid (CAS Number: 20283-92-5), 4'.5.7 trihydroxyflavanone (CAS Number: 67604-48-2), 3',5,7 trihydroxy-4'-methoxyflavone (CAS Number:520-33-2). And the blend four including: 3-hydroxybenzoic acid (CAS Number: 99-06-9), 4-hydroxybenzoic acid (CAS Number: 99-96-7), 3,4-dihydroxycinnamic acid (CAS Number: 331-39-5), 4-hydroxy-3,5-dimethoxycinnamic acid (CAS Number: 530-59-6), ellagic acid (CAS Number: 476-66-4), tannic acid (CAS Number: 1401-55-4), 3',4',5,7-tetrahydroxyflavone (CAS Number: 491-70-3), curcumin (CAS Number: 458-37-7). Reactif Folin-Ciocalteu (CAS Number: 12111-13-6), 2,2-Diphenyl-1-picrylhydrazyl (CAS Number: 1898-66-4), and Ascorbic acid (CAS Number: 50-81-7, Sigma).

2.3. Determination of total phenolics, total flavonoids and tannins

The polyphenols were determined by the Folin-Ciocalteu method according to Li *et al*. [10]. The

results were reported in milligrams of gallic acid equivalent per gram of fresh weight (mg GE g^{-1} FW). The flavonoids were characterized by the aluminum trichloride ($AlCl_3$) method according to Shraim researcher [11]. Results were expressed as micrograms of quercetin equivalent per gram of fresh weight (mg QE g^{-1} FW). The condensed tannins were determined by the vanillin acid method [12]. Results were expressed as milligrams of catechin equivalent per gram of fresh weight (mg CE g^{-1} FW).

2.4. Analysis of DPPH radical scavenging activity

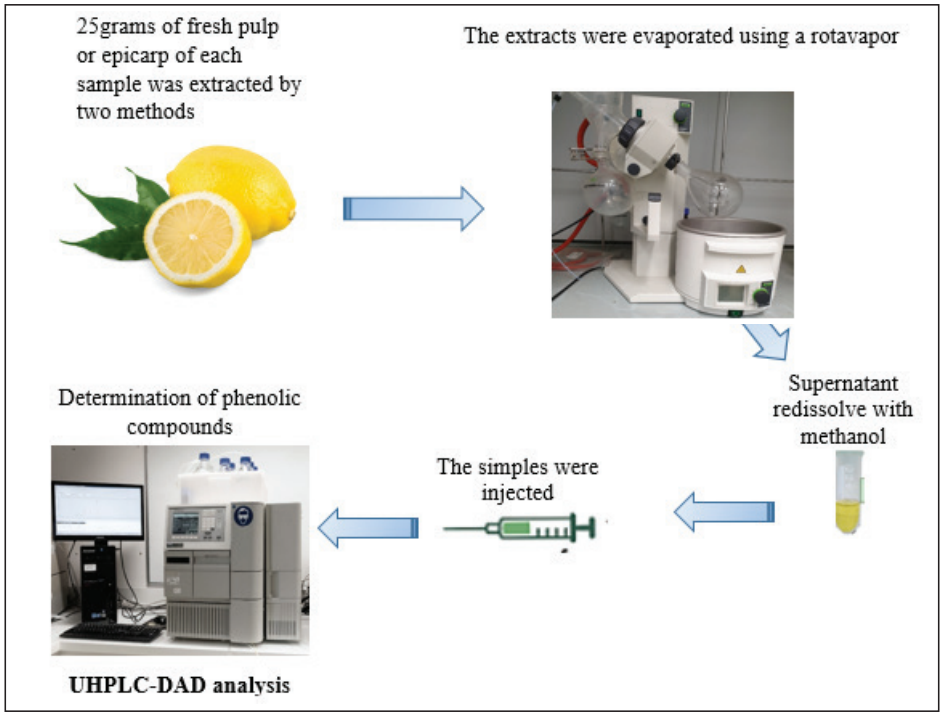
According to Awika et al. [13], antioxidant activity was measured using the DPPH method. The methanol dissolved the DPPH radical at a 5.5 mg mL^{-1} concentration. Then, it was sonicated for 3 minutes and kept at -4 °C for 1 hour at obscurity. Briefly, 1.0 mL of each extract of pulp or epicarp was added to 2.5 mL of DPPH solution, and the absorbance was measured after 30 min at 515 nm. DPPH trapping was determined using Equation 1.

$$IC50\% = [1 - (Abs\ (t\ =\ 30)/Abs\ (t = 0))] * 100 \quad \text{(Eq. 1)}$$

Abs (t = 0): absorbance of the *DPPH* radical at t = 0 min
Abs (t = 30): absorbance of the *DPPH* radical + phenolic extracts at t = 30 min.
The positive control was a solution containing the standard antioxidants: ascorbic acid, gallic acid, and quercetin. The absorbance of the solution was evaluated under identical conditions as the samples. For each concentration, the test was repeated three times.

2.5. Determination of phenolic compound

The Dionex Ultimate 3000 chromatography system (CA, USA), which is furnished with a quaternary pump (HPG 3400 RS), an autosampler (WPS 3000 TSL) and a column oven (TCC 3000), was used to carry out the chromatographic separation. A Kinetex C18 reversed phase column (250 × 4,6 mm, Eurospher 100-5) provided by Thermo Fisher Scientific (CA, USA) was used for the proposed technique. The separation and identification of phenolic compounds was performed using the method of Puigventós et al. [14]. Retention times and UV spectra were compared to standards to identify the compounds investigated in the methanolic and ethyl acetate extracts (Schema 1).



Schema 1. Schematic of study stages including the sampling, the extraction method, and analysis of phenolic compounds by UHPLC-DAD

Also, the Chromatographic profile of pulp from methanolic extracts of C. limon (Cp1), C. limetta (Cp2) and C. aurantifolia (Cp3) by two extraction methods maceration M and soxhlet S were shown in Figure 1a. Chromatographic profile of epicarp from methanolic extracts of C. limon (Cz1), C. limetta (Cz2) and C. aurantifolia (Cz3) by two extraction methods maceration M and soxhlet S were shown in Figure 1b. Chromatographic profile of pulps from ethyl acetate extracts of C. limon (Cp1), C. limetta (Cp2) and C. aurantifolia (Cp3) by two extraction methods, maceration M and soxhlet S, were shown in Figure 1c. Chromatographic profile of epicarp from methanolic extracts of C. limon (Cz1), C. limetta (Cz2) and C. aurantifolia (Cz3) by two extraction methods maceration M and soxhlet S. was shown in Figure 1d. The chromatographic profile of the multi-standards is shown in Figure 1e and Table 1.

Table 1. The multi standards with their retention times

Compounds	Retention time
Blend 1	
Pyrogallic Ac	5.45
Vanillic Ac	11.81
Cafeic Ac	12.18
Furelic Ac	16.51
Hesperidin	18.76
Salicyclic Ac	19.48
Blend 2	
Gallic Ac	5.86
Catechin	9.44
Chlorogenic ac	10.87
Epicathechin	12.13
vanillin	12.72
p-coumaric	15.41
Sinapic Ac	16.87
Naringin	19.04
Rutin	20.31
Quercetin	24.84
Kaempferol	26.82
Blend 3	
Catechol	8.07
Hydroxybezoic Ac	10.76
Syringic Ac	12.54
dimethoxybezoic Ac 3,4	16.46
hydroxynaphthoic Ac 2	19.20
Rosmarinic Ac	20.14
trihydroxyflavanon 4,5,7	24.05
trihydroxy-4'-methoxyflavon 5,7,'3	25.34
Blend 4	
hydroxybenzoic Ac-3	7.74
hydroxybenzoic Ac-4	10.76
dihydroxycinnamic Ac 3,4	12.20
hydroxy-3,5-dimethoxynnamic-4	16.90
Ellagic Ac	21.74
Tannic Ac	24.87
tetrahydroxyflavon-5,7,'4,'3	26.15
Curcumin	28.16

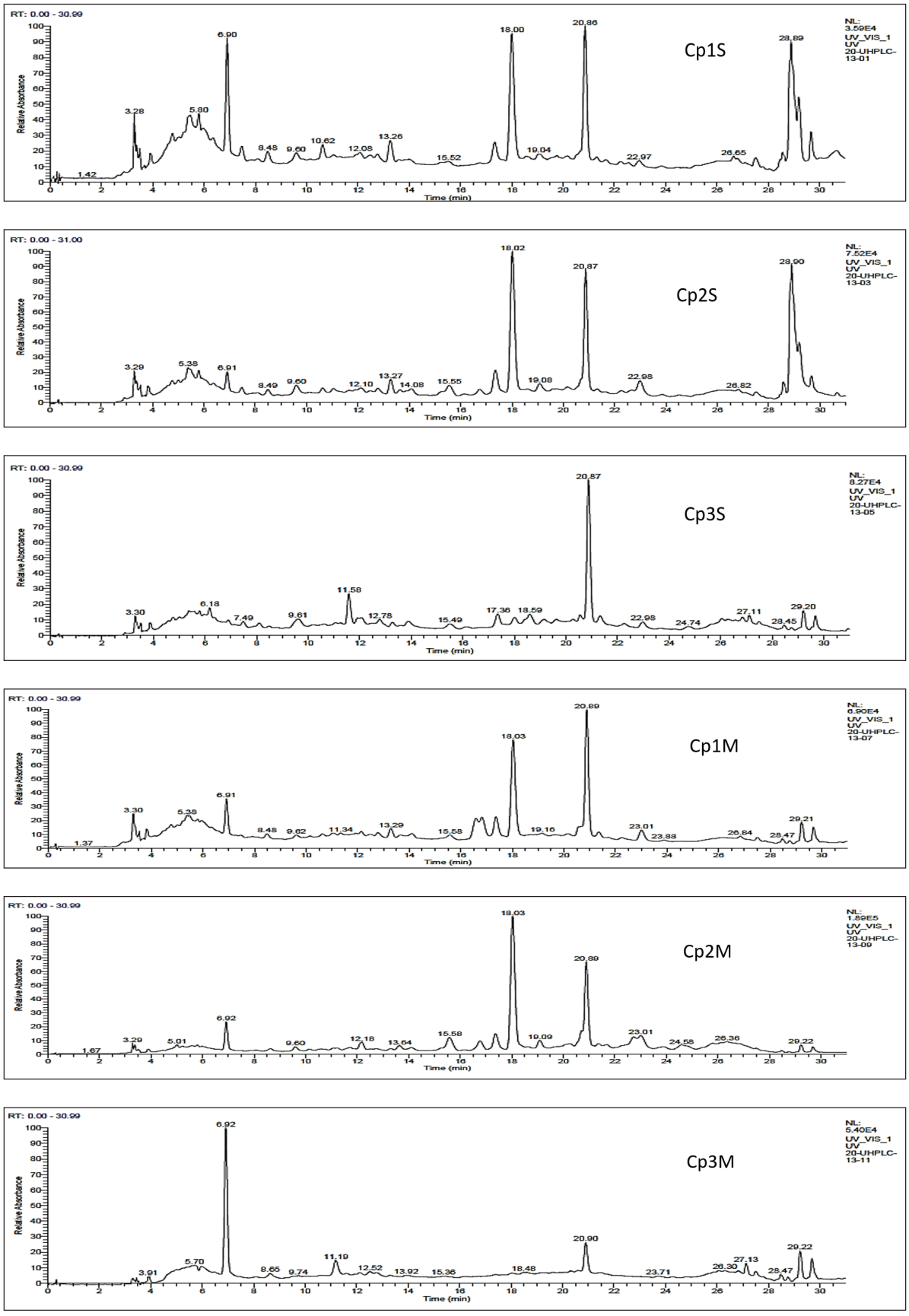


Fig. 1a. Chromatographic profile of pulp from methanolic extracts of *C. limon (Cp1), C. limetta (Cp2) and C. aurantifolia (Cp3)* by two extraction methods maceration M and soxhlet S.

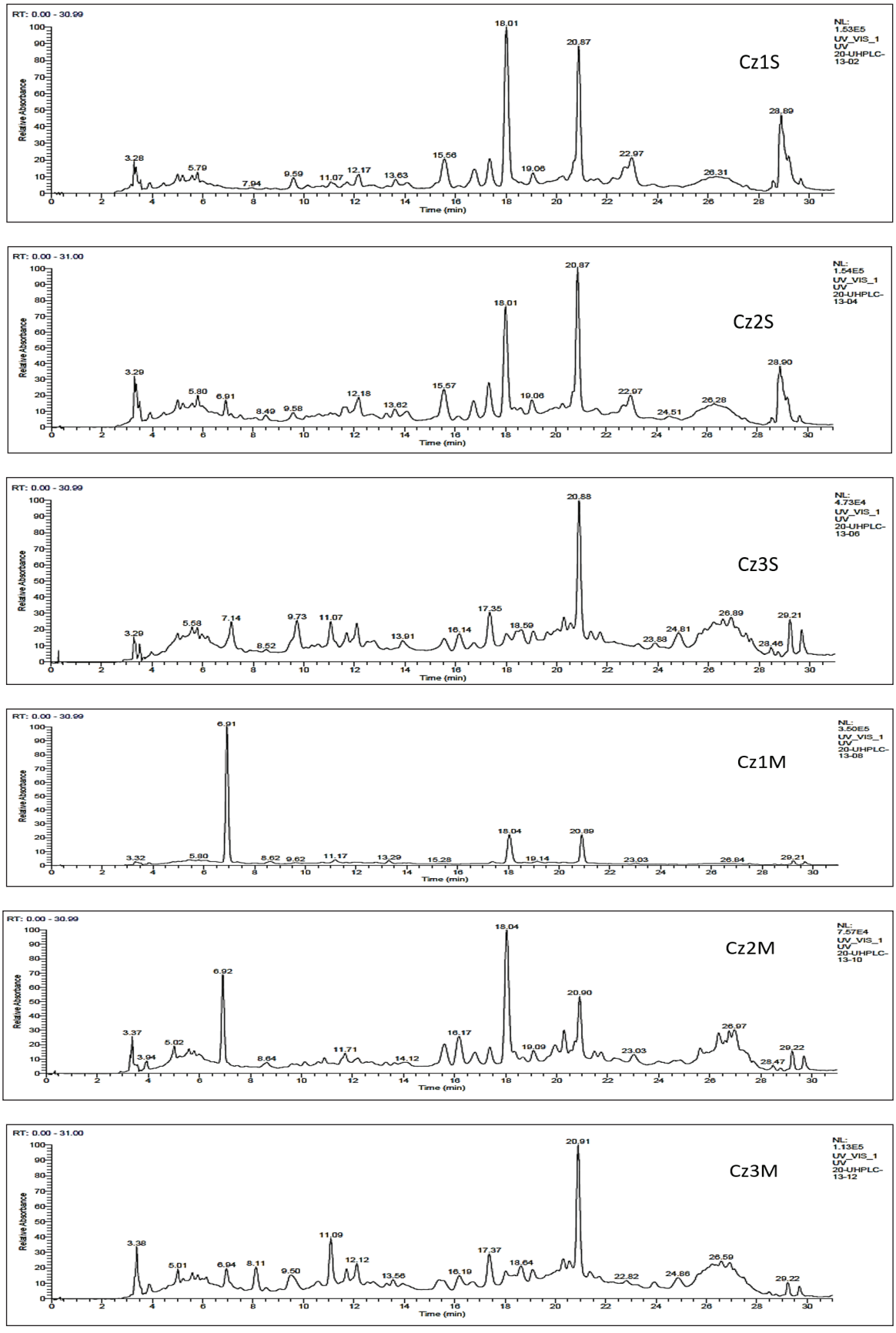


Fig. 1b. Chromatographic profile of epicarp from methanolic extracts of *C. limon (Cz1), C. limetta (Cz2) and C. aurantifolia (Cz3)* by two extraction methods maceration M and soxhlet S.

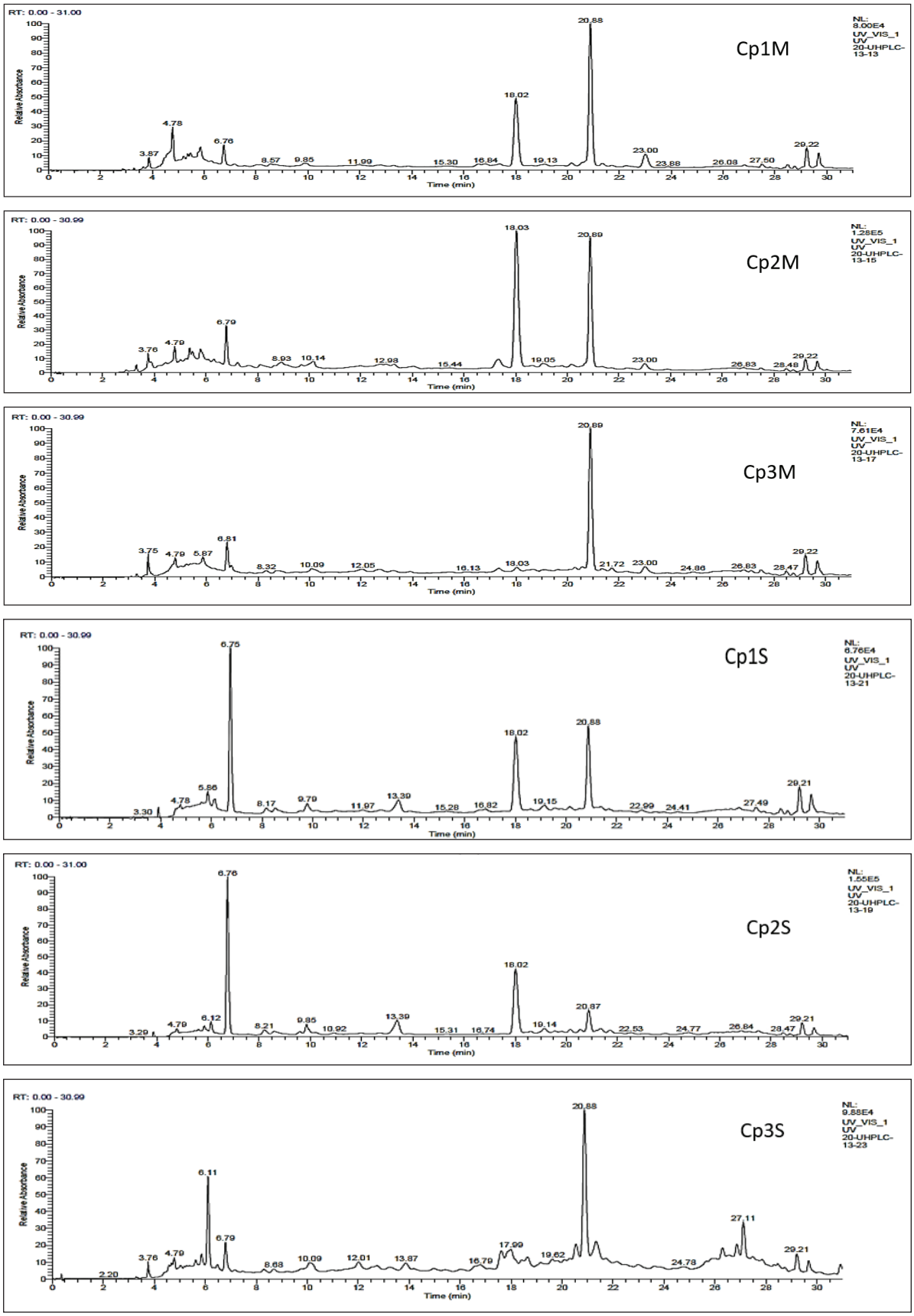


Fig. 1c. Chromatographic profile of pulps from ethyl acetate extracts of *C. limon (Cp1), C. limetta (Cp2) and C. aurantifolia (Cp3)* by two extraction methods maceration M and soxhlet S

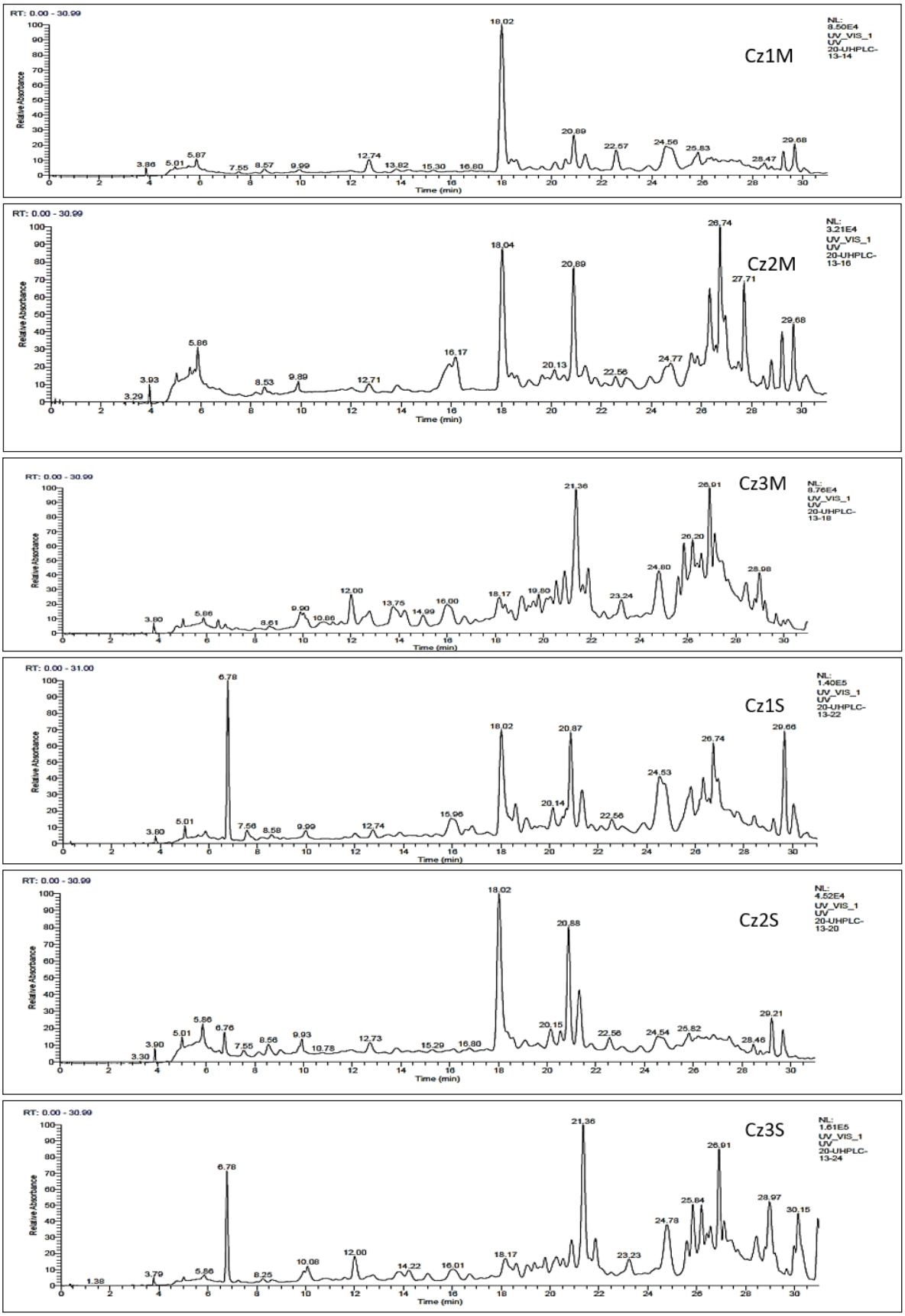


Fig. 1d. Chromatographic profile of epicarp from methanolic extracts of *C. limon (Cz1), C. limetta (Cz2) and C. aurantifolia (Cz3)* by two extraction methods maceration M and soxhlet S.

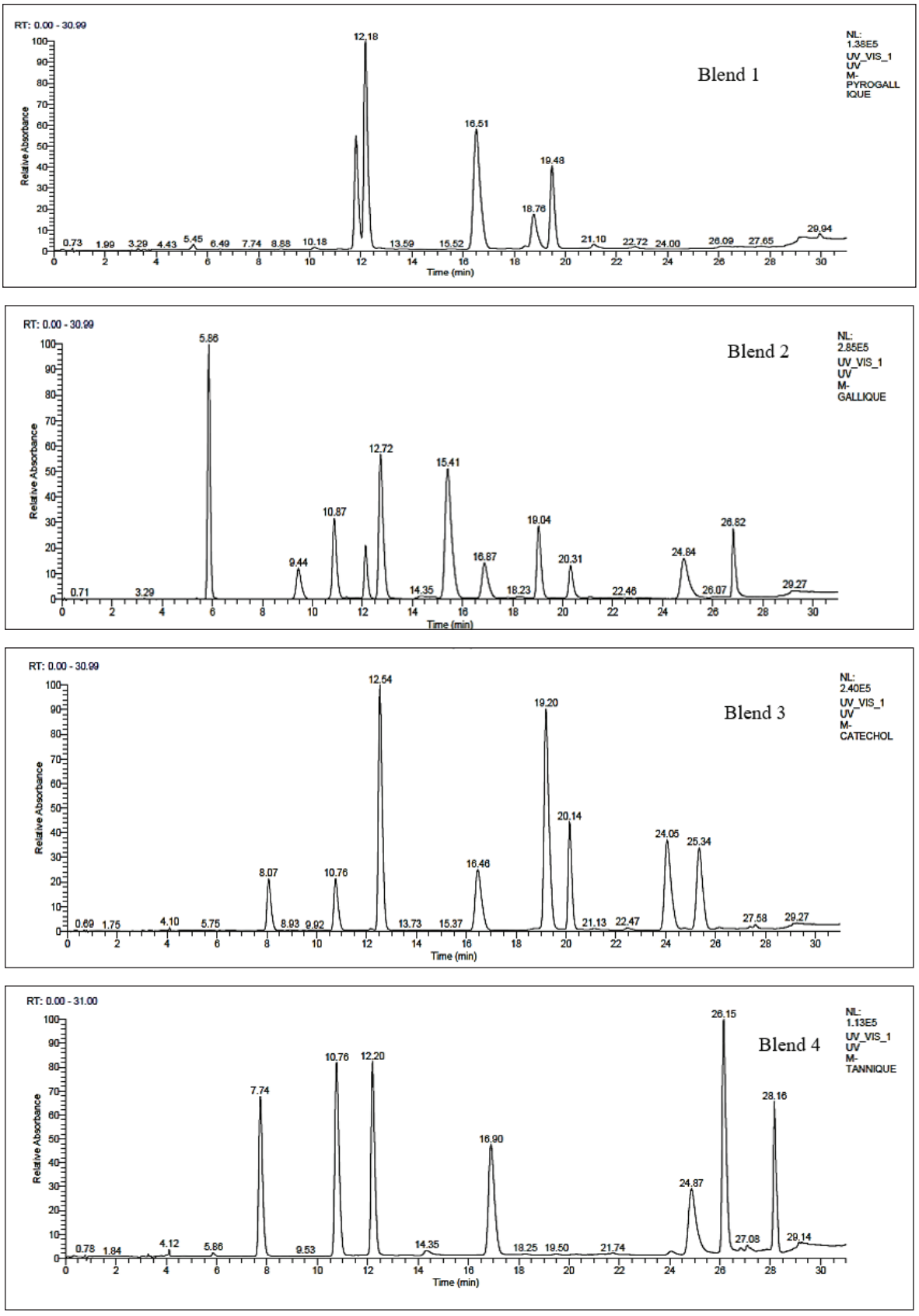


Fig.1e. Chromatographic profile of multi standards

2.6. Statistical Analysis

Data were subjected to a two-factor analysis of variance (*ANOVA*) using the statistical software SPSS for windows (version 20.0, IBM-SPSS Inc., Chicago, IL, USA). Significant differences among treatments were determined using the student-Newman-Keuls (*SNK*) post-hoc test. Mean data followed by different letters are significantly different at $p<0.05$.

3. Results and Discussion

3.1. Crude extracts yield

The edible part*:* The result in Figure 2 showed that the Soxhlet extraction gave higher yields than the maceration method. For both extraction methods, the best yield was obtained with ethyl acetate in *C. limon* (*Cp1*) followed by *C. limetta* (*Cp2*) and *C. aurantifolia* (*Cp3*). At the same time, the lowest yield was with hexane in all three species. The non-edible part*:* We found that the yields of soxhlet extraction varied considerably compared to the yields by maceration (Fig. 3). The recorded percentages ranged from 0.62% to 12.46%. The methanol gave the best yield in *C. limetta* (*Cz2*) followed by *C. limon* (*Cz1*) and finally by *C. aurantifolia* (*Cz3*). On the other hand, the yield of dichloromethane was the lowest for all three species.

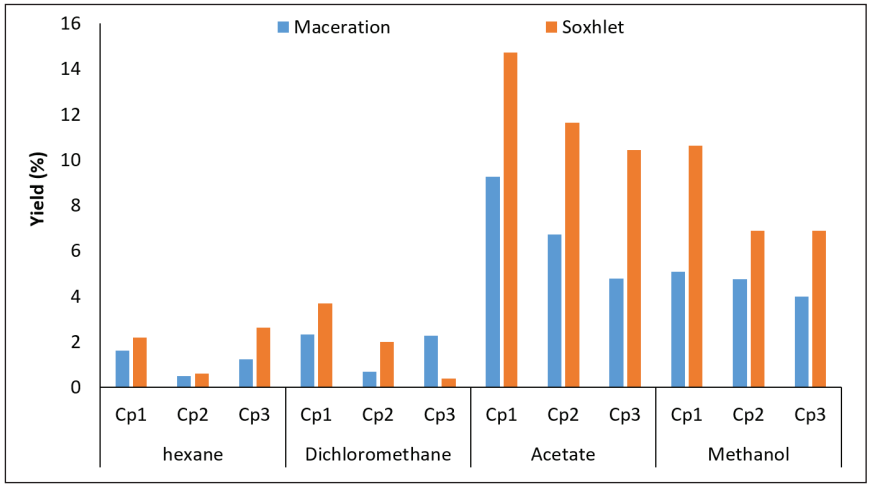


Fig. 2. Crude pulp extract yield (%) of *C. limon (Cp1), C. limetta (Cp2), C. aurantifolia (Cp3)* by two extraction methods (maceration, soxhlet) and four solvents (hexane, dichloromethane, ethyl acetate, methanol). Data are means of 3 replicates ± standard deviation. Different letters above the bars represent a significant difference ($p < 0.05$) between treatments according to Student-Newman-Keuls test.

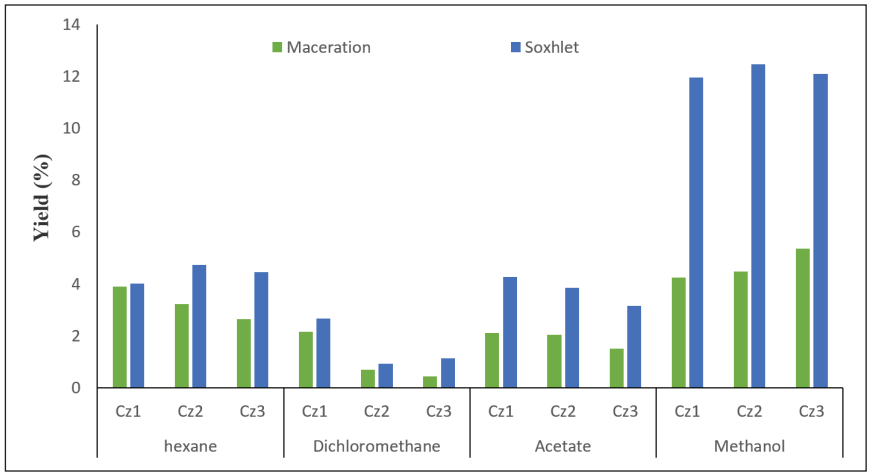
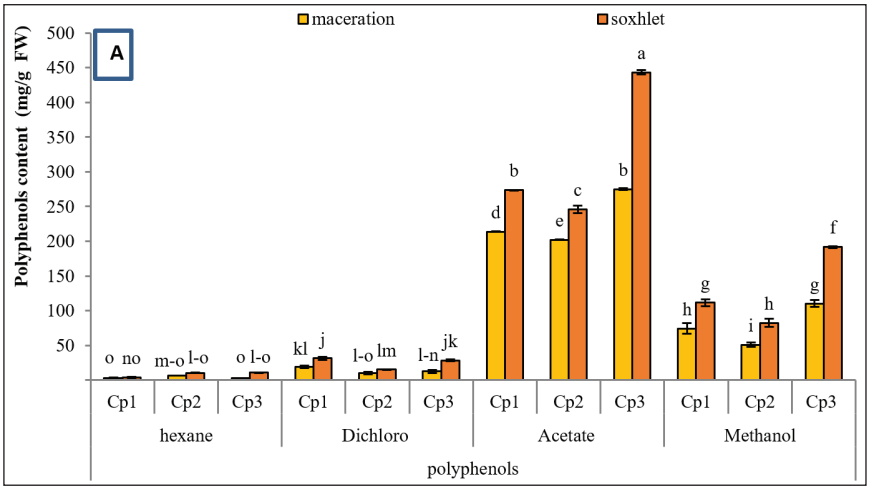
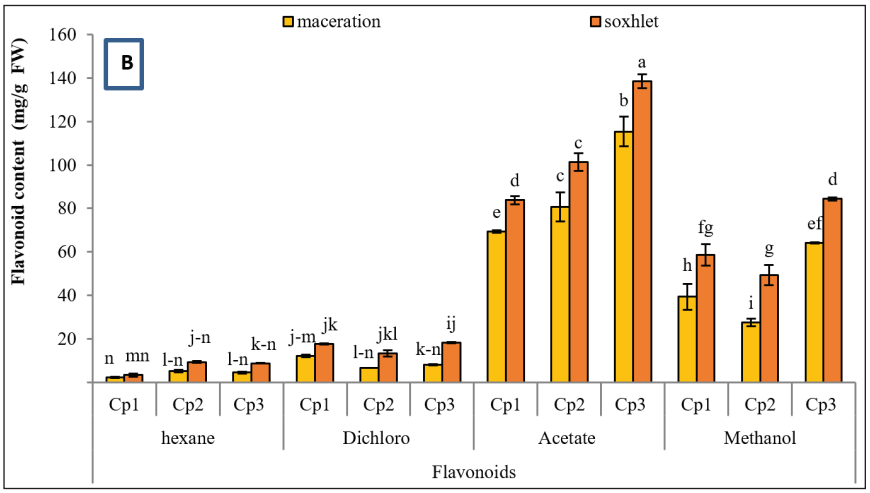


Fig. 3. Crude epicarp extract yield (%) of *C.limon (Cz1), C.limetta (Cz2), C.aurantifolia (Cz3)* by two extraction methods (maceration, soxhlet) and four solvents (hexane, dichloromethane, ethyl acetate, methanol). Data are means of 3 replicates ± standard deviation. Different letters above the bars represent a significant difference ($p < 0.05$) between treatments according to Student-Newman-Keuls test.

3.2. The effect of solvents and extraction methods on phenolic components

Figures 4 and 5 present the results of a quantitative comparison of total phenols, flavonoids, and condensed tannins in the pulp and epicarp parts of three studied citrus species. The edible part: in Figure 4, the results related to the amounts of total phenols, flavonoids, and condensed tannins in the extracts of the pulp of the three species studied showed that Soxhlet extraction as a method and ethyl acetate as a solvent gave the best results. The results obtained in Figure 4A showed that Citrus aurantifolia contained the highest polyphenol content (443.57±3.10 mg g^{-1} FW), followed by Citrus limon (273.75±0.43 mg g^{-1} FW) and, at the end, *Citrus limetta* (245.81±5.58 mg g^{-1} FW). Regarding flavonoid content, it was higher in *Citrus aurantifolia* (138.62±3.21 mg g^{-1} FW), followed by *Citrus limetta* (101.34±9.56 mg g^{-1} FW) and by *Citrus limon* (83.85±1.86 mgg^{-1} FW) (Fig.4B). In contrast, the condensed tannin contents obtained in *Citrus limetta, Citrus aurantifolia and Citrus limon* are 46.90±1.15 mgg^{-1} FW, 46.32±6.76 mg g^{-1} FW and 44.68±5.95 mgg^{-1} FW, respectively (Fig.4C). The non-edible part: in Figure 4, the results related to the amounts of total phenols, flavonoids, and condensed tannins in extracts of the epicarp of the three species studied showed that Soxhlet extraction as a method and methanol as a solvent gave the best results. The results of the assay revealed that the highest amounts of total phenols were recorded in *Citrus aurantifolia* with 326.73±9.82 mgg^{-1} FW, followed by *Citrus limetta* with 229.08±8.59 mgg^{-1} FW and by *Citrus limon* with 183.16±1.40 mgg^{-1} FW (Fig.5A). The flavonoid content was abundant in *Citrus aurantifolia* (133.00±3.55 mgg^{-1} FW) followed by *Citrus limetta* (104.59±4.54 mgg^{-1} FW) and by *Citrus limon* (81.19±8.19 mgg^{-1} FW) (Fig.5B). Concerning, the condensed tannin contents found in *Citrus limetta, Citrus aurantifolia* and *Citrus limon*, we note 49.19±12.17 mg g^{-1} FW, 31.34±1.15 mgg^{-1} FW and 43.86±0.00 mgg^{-1} FW respectively (Fig.5C).





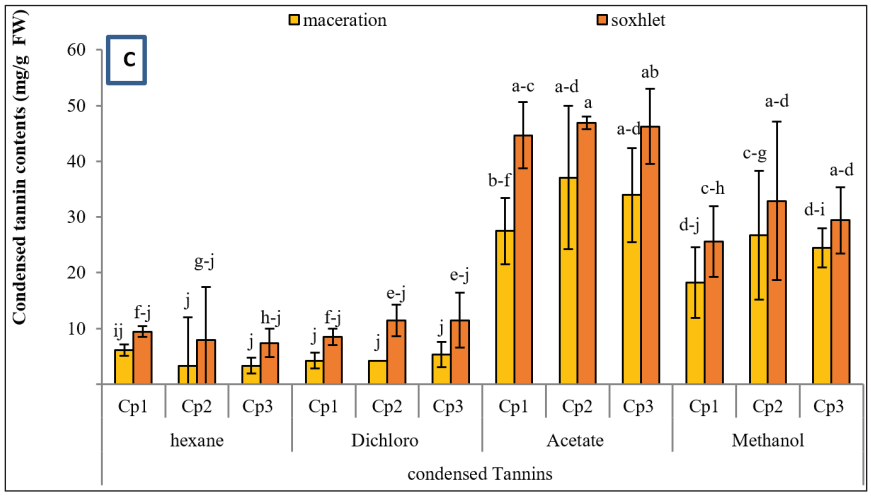
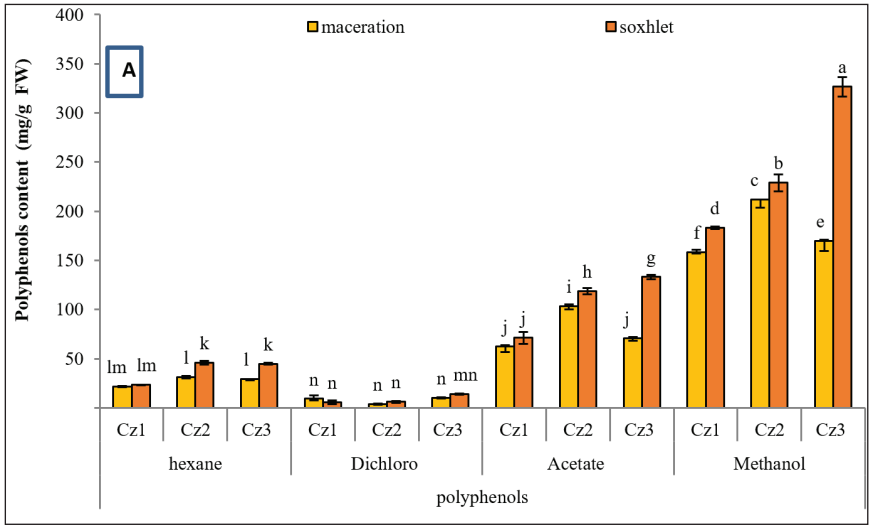
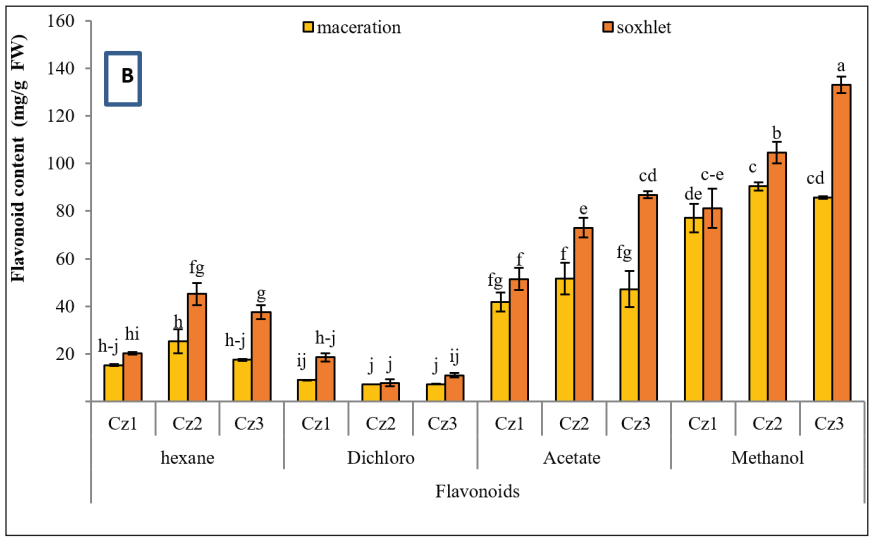


Fig. 4. The polyphenol (A), flavonoid (B) and condensed tannin (C) content of *Citrus Limon Burm (Cp1), Citrus Limetta Risso (Cp2)* and *Citrus Aurantiifolia* (Christm.) Swingle (Cp3) pulps by two extraction methods (maceration, soxhlet) and four solvents (hexane, dichloromethane, ethyl acetate and methanol). Data are means of 3 replicates ± standard deviation. Different letters above the bars represent a significant difference ($p < 0.05$) between treatments according to Student-Newman-Keuls test.





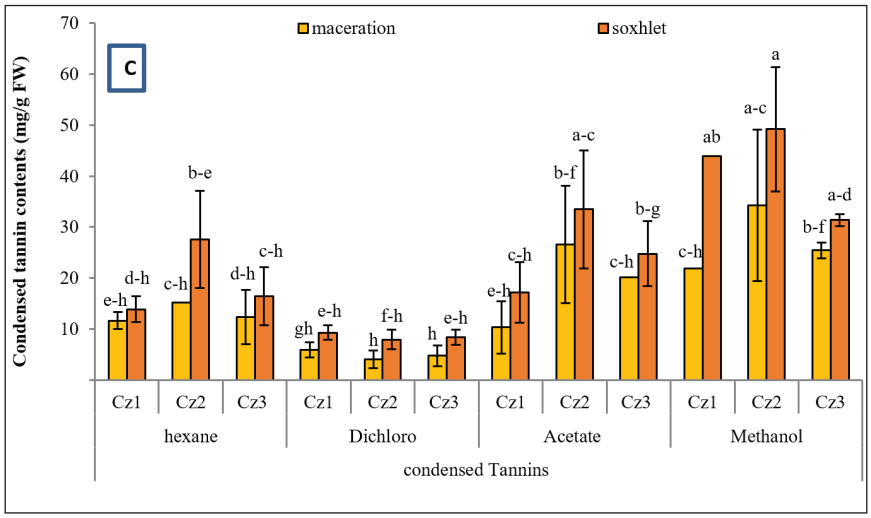


Fig. 5. The polyphenol (A), flavonoid (B) and condensed tannin (C) content of *Citrus Limon Burm* (*Cz1), Citrus Limetta Risso* (*Cz2*) and *Citrus Aurantiifolia (Christm.) Swingle* (*Cz3*) epicarp by two extraction methods (maceration, soxhlet) and four solvents (hexane, dichloromethane, ethyl acetate, methanol). Data are means of 3 replicates ± standard deviation. Different letters above the bars represent a significant difference ($p < 0.05$) between treatments according to Student-Newman-Keuls test

3.3. The effect of solvents and extraction methods on the antioxidant activity (DPPH)

The edible part: from the results obtained on the pulps, statistical analysis showed that the solvents used presented a significant difference ($p<0.05$). Thus, the profiles of the *DPPH*- test obtained revealed that all the extracts tested possessed dose-dependent antiradical activity (Fig. 6). The ethyl acetate extracts showed a high inhibition of the *DPPH*- radical very close to those of the control solutions (gallic acid, quercitin and ascorbic acid); 4.89±0.15 µg ml^{-1} for *Citrus aurantifolia*, 9.54±0.15 µg ml^{-1} for *Citrus limon* and 9.74±0.22 µg ml^{-1} for *Citrus limetta*, followed by the methanol extracts. On the other hand, a very low antiradical activity was noted in hexane and dichloromethane extracts. Regarding the extraction method, the antiradical activities of the analyzed samples showed a significant difference ($p<0.05$). The results obtained showed that the extracts obtained by Soxhlet have the highest antioxidant capacity compared to that of maceration, regardless of the extraction solvent. For comparative purposes, three standard antioxidants were used, gallic acid, quercitin and ascorbic acid (Fig. 6). They showed a high free radical scavenging activity with IC_{50} values in the range of 7.80 ± 0.25 µg ml^{-1}, 4.06 ± 0.26 µg ml^{-1} and 2.48 ± 0.32 µg ml^{-1}, respectively. The lower *the IC50 value, the more potent the extract was considered* an antioxidant. The non-edible part*:* the antiradical activities of the analyzed samples revealed significant differences ($p<0.05$) (Fig.7). We also found that almost all the extracts studied have antioxidant activity and were able to scavenge the *DPPH* radical. We noted that the extracts that showed the best inhibitory activity were the methanolic and ethyl acetate extracts, with IC50 values that vary from 5.13 µg.ml^{-1} to 31,13 µg.ml^{-1}. However, the highest free radical scavenging activity was observed in the methanolic extracts of *Citrus autantifolia* (5.13±0.12 µg ml^{-1}) followed by *Citrus limetta* (6.16±0.10 µg ml^{-1}) and *Citrus limon* (10.78±0.12 µg.ml^{-1}). The activity of these extracts evolves in the same direction as those of gallic acid with 7.80±0.25 µg.ml^{-1} and quercitin with 4.06±0.26 µg ml^{-1}, while ascorbic acid showed the highest antiradical activity with 2.48±0.32 µg.ml^{-1}. On the other hand, the lowest antiradical activity was obtained with hexane and dichloromethane (57.24 µg ml^{-1} and 138.70 µg ml^{-1}, respectively).

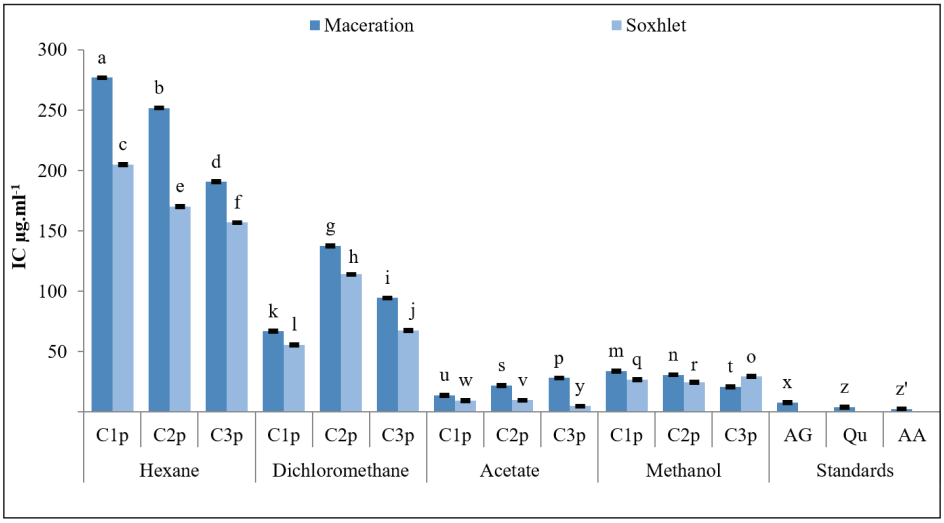


Fig. 6. Antiradical activity of *Citrus Limon Burm* (*Cp1*), *Citrus Limetta Risso* (*Cp2*) and *Citrus Aurantiifolia (Christm.) Swingle* (*Cp3*) pulp by two extraction methods (maceration, soxhlet) and four solvents (hexane, dichloromethane, ethyl acetate, methanol). Data are means of 3 replicates ± standard deviation. Different letters above the bars represent a significant difference ($p < 0.05$) between treatments according to Student-Newman-Keuls test.

3.4. Determination of phenolic compound by *UHPLC-DAD*

In this study, a method of high-performance liquid chromatography (*UHPLC*) coupled to a diode array detector (*DAD*), was used for the characterization of the phenolic compounds of the methanolic and ethyl acetate extracts of the edible and non-edible par of three *Citrus* species studied (*Citrus Limon Burm, Citrus Limetta Risso and Citrus Aurantiifolia (Christm.) Swingle)* for the two extraction methods (maceration and soxhlet). Thirty-three standards were divided into 4 blends to identify the different metabolites. Fifty-five phenolic compounds were tentatively identified in the polar *Citrus* pulp and epicarp extracts based on their maximum wavelength UV absorption and using the peaks of the standards. Among them, the identification of three flavonols (rutin, kaempferol and quercetin), two flavanones (hesperidin and naringin), a flavone (3', 4', 5, 7-tetrahydroxyflavone) and a phenolic acid (pyrogallic acid) which have been confirmed alongside literature data on the chemical composition of *Citrus*. The edible part*:* the three *Citrus* studied contained two flavonoids, of which hesperidin (6.62 g L^{-1}) was the highest in the methanolic extract of *C. limetta* by maceration, followed by rutin (2.52 g L^{-1}) in the ethyl acetate extract of *C. limetta* by maceration, then a phenolic acid, whose pyrogallic acid (1.48 g L^{-1}) was moderately high in the methanolic extract of *C. limetta* by Soxhlet. While the other metabolites (Gallic, Catechol, Catechin, Pcoumaric, Naringin, Ac hydroxybezoic, Sinapic, 3', 4', 5, 7 - tetrahydroxyflavone, Kaempferol, Quercetin, 3',5,7 trihydroxy-4'-methoxyflavone and Epicathechin) were present at trace levels (Table 2). The non-edible part*:* in the ethyl acetate and methanol extracts of *C. limetta* by Soxhlet, high contents of hesperidin (4.34 g L^{-1}) and rutin (3.39 g L^{-1}) were recorded, followed by pyrogallic acid (1.79 g L^{-1}) in the methanolic extract of C. aurantifolia by Soxhlet and then naringin (1.77 g L^{-1}) in the methanolic extract by Soxhlet of *C. limon*. On the other hand, the other metabolites presented in trace amounts (Table 3). According to these results, we suggest that the phenolic compounds content in the pulps of three species was higher than that of the epicarp. The highest concentration of phenolic compounds in juice is generally due to the very hydrophilic nature of these components.

Table 2. Provisional identification of phenolic compounds of the edible part of *Citrus Limon Burm* (*Cp1*), *Citrus Limetta Risso* (*Cp2*) and *Citrus Aurantiifolia* (*Cp3*) by *UHPLC-DAD*.

Extraction methods	Solvent	PA	GA	C	Ca	P-C	Nar	Hes	Rut	HbA	Sin	THF	Kaem	Quer	TMF	Epi
Maceration		0.51	0.04	0.02	0.03	----	0.01	1.36	1.72	----	0.02	0.01	----	----	----	0.02
	acetate	1.17	0.06	0.16	0.05	0.01	0.06	4.40	2.52	----	----	----	0.05	----	----	----
		0.15	0.03	0.03	----	----	----	0.08	----	----	----	----	0.02	0.01	----	----
		0.93	0.02	0.04	0.05	0.02	0.02	1.78	1.5	----	0.2	0.04	0.05	----	----	----
	Methanol	----	----	----	0.15	0.14	0.14	6.62	1.63	----	0.31	0.21	0.20	0.13	----	----
		0.4	0.01	0.04	0.01	----	----	----	0.25	----	----	0.02	0.03	----	----	----
Soxhlet		----	0.03	0.11	0.22	----	0.06	2.33	0.53	0.03	0.01	----	0.05	0.06	----	----
	Acetate	----	0.03	0.05	0.09	----	0.03	1.09	0.77	----	0.02	----	0.05	----	----	----
		0.59	0.03	0.04	----	----	----	0.18	0.06	0.07	----	0.05	0.09	0.04		0.11
		1.48	0.02	0.05	0.07	0.01	----	1.11	0.75	0.06	----	----	----	----	----	----
	Methanol	1.17	0.03	0.05	0.14	0.04	0.05	2.60	1.38	----	----	0.05	----	----	0.01	----
		0.53	0.02	0.06	0.18	0.02	0.04	0.27	1.74	0.05	----	----	----	----	----	----

*(PA) Pyrogallic Acid, (GA) Gallic Acid, (C) Catechol, (Ca) Catechin, (P-C) p-coumaric, (Nar) Naringin, (Hes)Hesperidin, (Rut) Rutin, (HbA) Hydroxybezoic Acid, (Sin) Sinapic Acid, (THF) 3',4',5,7-tetrahydroxyflavone, (Kaem) Kaempferol, (Quer) Quercetin, (TMF) 3',5,7 trihydroxy-4'methoxyflavone, (Epi) Epicathechin.

Table 3. Provisional identification of phenolic compounds of the non-edible part of Citrus Limon Burm (Cz1), Citrus Limetta Risso (Cz2) and Citrus Aurantiifolia (Cz3) by UHPLC-DAD

Extraction methods	Solvent	species	PA	GA	C	Ca	P-C	Nar	Hes	Rut	HbA	Sin	THF	Kaem	Quer	TMF	Epi
Maceration	acetate	Cz1	1.13	0.04	0.05	0.05	0.01	----	3.01	0.14	0.02	0.01	----	----	0.45	----	----
		Cz2	----	0.02	0.02	0.04	0.04	----	1.03	0.47	----	----	0.12	0.31	0.12	0.05	----
		Cz3	----	0.03	----	0.02	0.04	0.21	0.57	0.32	0.09	----	0.33	0.62	0.67	0.15	0.38
	Methanol	Cz1	0.81	0.02	0.16	----	0.01	0.04	2.7	1.63	----	----	0.07	0.06	----	----	----
		Cz2	0.73	----	0.08	----	0.07	0.07	2.61	0.76	----	0.16	0.09	0.13	----	----	----
		Cz3	1.66	0.03	0.35	0.47	0.04	0.19	----	2.37	----	----	0.23	0.25	----	----	0.34
Soxhlet	Acetate	Cz1	0.58	0.03	0.07	0.09	----	0.04	1.71	0.72	0.03	0.02	----	----	0.13	----	----
		Cz2	1.02	0.03	0.08	0.19	0.15	0.18	4.34	1.83	0.18	----	0.21	0.64	0.55	----	----
		Cz3	----	0.04	0.08	----	----	0.17	0.51	0.69	----	----	0.33	1.12	1.11	0.18	----
	Methanol	Cz1	----	0.03	----	0.28	0.19	1.77	1.03	0.31	----	0.43	1.04	0.27	----	0.05	----
		Cz2	----	0.05	0.11	0.22	0.19	0.17	3.95	3.39	----	0.4	0.41	----	0.1	0.08	----
		Cz3	1.79	0.03	0.01	0.29	0.02	0.04	0.16	0.94	0.16	----	0.09	0.13	----	----	----

*(PA) Pyrogallic Acid, (GA) Gallic Acid, (C) Catechol, (Ca) Catechin, (P-C) p-coumaric, (Nar) Naringin, (Hes)Hesperidin, (Rut) Rutin, (HbA) Hydroxybezoic Acid, (Sin) Sinapic Acid, (THF) 3',4',5,7-tetrahydroxyflavone, (Kaem) Kaempferol, (Quer) Quercetin, (TMF) 3',5,7 trihydroxy-4'methoxyflavone, (Epi) Epicathechin.

3.5. Discussion

The main objective of this study was to assess *in vitro* the influence of solvents and extraction methods on the phenolic composition and antioxidant activity of three *Citrus* species extracts using the *DPPH* free radical scavenging method. The data collected demonstrated that each solvent's yield of the crude extracts differed depending on the extraction method and species (0.40% to 14.72%). Similarly, Ibrahim and Hegazy [15] found that the yield of orange epicarp extracts using various solvents ranged from 8.27% to 28.32%. The samples' nature and differences in solvent diffusion explained this variation. It was also due to several factors, including the interaction of the plant with the environment (climate, soils, etc.), the time and place of harvest, cultural practices, and the age of plant material [16]. Maceration and Soxhlet are considered the conventional phenolic compound extraction methods approved by various authors for their efficiencies [17]. Our results showed that the extraction method significantly influences the yield and phenolic compound contents in *citrus limon, citrus aurantifolia, and citrus limetta.* Soxhlet extraction allowed an enrichment in phenolic compounds compared to maceration. Similarly, several works have presented similar results, which indicated a significant variation in the contents of these compounds depending on the technique used [18]. In another study, the Lebanese Eryngium Creticum was extracted using three conventional techniques: maceration, reflux, and Soxhlet. The results showed that among these techniques, reflux and Soxhlet gave approximately the same results compared to maceration [19]. Thus, a comparison between the two methods showed that soxhlet remains the preferred method for extraction [20]. Similarly, Teimoori et al [21] have suggested other extraction methods based on ultrasonic dispersive solid-phase micro-extraction. In our study, solvents of increasing polarity (hexane, dichloromethane, ethyl acetate, and methanol) were used to extract three *Citrus* species, to have as many secondary metabolites of different natures as possible and to separate these metabolites according to their degree of solubility in the solvent. Thus, their degree of glycosylation mainly depends on the number of hydroxyl groups, molecular weight and carbon chain length [22]. Indeed, the hexane extract is generally composed of lipids and highly methoxylated aglycone flavonoids, the dichloromethane extract is richer in aglycone flavonoids, ethyl acetate extract contains glycosylated flavonoids, mainly mono-, di- and tri-glycosylated flavonoids, and the methanolic extract contains flavonoids, amino acids, terpenes, waxes and tannins [23]. According to our results, the quantification of polyphenols, flavonoids and condensed tannins by different solvents showed that methanol and ethyl acetate extracts presented high contents of these molecules compared to hexane and dichloromethane extracts. Ghasemzadeh *et al*. [24] and Barchan *et al*. [25] worked on three species of Mantha from Morocco and two varieties of young ginger from Malaysia, respectively. They also showed that polar solvents (methanol, acetone) yielded higher phenolic compounds than apolar solvents (hexane, dichloromethane, and chloroform). However, other studies reported by Mendes Harcke *et al*. [26], in *Gac* fruit respectively, found that ethyl acetate extracts exhibited high contents of phenolic compounds. Moreover, Al Juhaimi et al. [27] on methanolic and ethyl acetate extracts of some plants grown in Turkey revealed that both solvents showed almost the exact contents of phenolic compounds conducted a study. In this study, we noted that pulp and epicarp parts of the *Citrus* fruit accumulate the highest contents of polyphenols and flavonoids. The comparative analysis showed that *Citrus aurantifolia* was more nutritious than *Citrus limetta* and *Citrus limon* due to its relatively high content of polyphenols and flavonoids. These findings are consistent with those obtained by Khalfan Al-Musharf *et al*. [28] on six citrus species in Oman, who showed that *Citrus limon* juice presented a higher content of total phenols (0.569± 0.0019 mg EAG mL^{-1}) than *Citrus aurantifolia* (0.468± 0.0019 mg EAG mL^{-1}). Another study conducted in South India on the fruits of six *Citrus* species, revealed that *Citrus aurantifolia* possessed

a relatively high content of total phenols (125 µg EAG mg^{-1} for epicarp and 59.05µg EAG mg^{-1} for pulp) and flavonoids (36.49 µg EQ mg^{-1} for epicarp and 15.19 µg EQ mg^{-1} for pulp) [29]. In addition, Ghasemi *et al*. [30] reported that the fruit of *Citrus limon* from Iran, recorded relatively high content of polyphenols (131 mg EAG g^{-1} epicarp and 102.2 mg EAG g^{-1} pulp) and flavonoids. On the other hand, the *Citrus limetta* juice from Mexico showed high levels of polyphenols (786 ± 73.58 mg EAG.g^{-1}) and flavonoids (63.24 ± 3.66 EQ mg g^{-1}) [31]. In view of these results presented, it appears that the extraction of phenolic compounds is a crucial step for the valorisation of active ingredients. It depends on the extraction method, the solvent, the species and the nature of the studied part. From the results reported in Figures 6 and 7, we noticed that only polar extracts (methanol and ethyl acetate) by Soxhlet could inhibit the *DPPH* radical than apolar extracts (hexane and dichloromethane). Also, some other organic compounds were determined by nanotechnology coupled with a chromatography analyser [32-34]. Thus, for gallic acid, quercetin, and ascorbic acid, the percentage of *DPPH* inhibition was still high for these extracts. Because these extracts are rich in phenolic compounds (suitable donors of proton H+). Similarly, Kumaran and Joel, Karunakaran, [35] showed that polyphenols, flavonoids and condensed tannins can reduce and decolorize *DPPH* due to their ability to donate hydrogen. For example, Narayana *et al.* [36] found that flavonoids could inhibit the lipid peroxidation by scavenging free radicals. Indeed, the antioxidant activity is related to the structure of phenolic compounds. Thus, it depends on the O-H bonds' dissociation energy, the phenol radical's electronic delocalization, and the steric hindrance of the substituent groups of the hydrogen atoms of the aromatic ring [37]. The antioxidants influence biological systems by neutralizing free radicals and chelation of toxic metal [38]. Based on the results presented in Tables 1 and 2, ethyl acetate and methanol extracts can be considered the most interesting regarding antioxidant capacity, strongly related to the presence of phenols and polyphenolic compounds, such as flavonoids [39]. This led us to suggest that these extracts contain essential molecules that can serve as a source of natural antioxidants. These minor compounds give the three species important nutritional and therapeutic qualities. In addition, several authors have proposed other types of chromatography (GC-FID, GC-TCD, GC-MS) to identify these organic substances [40-43].

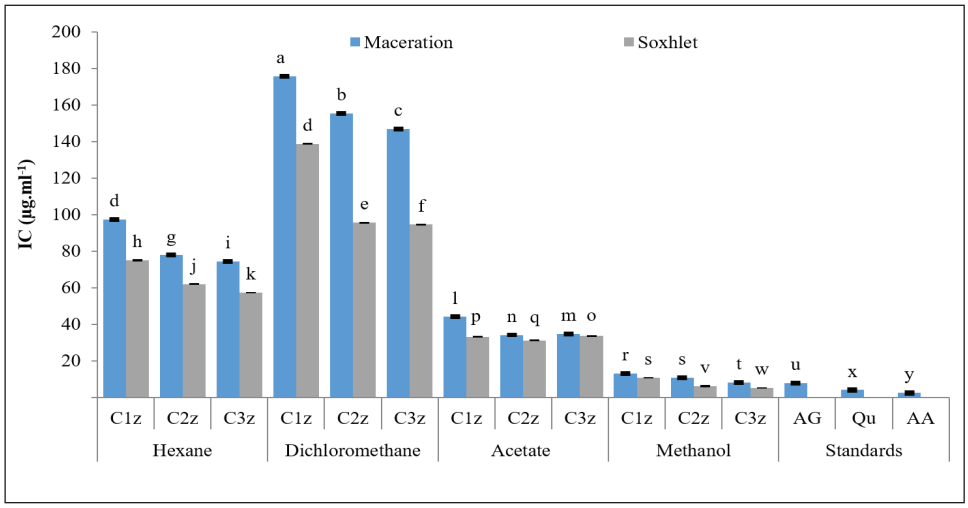


Fig. 7. Antiradical activity of *Citrus Limon Burm* (*Cz1*), *Citrus Limetta Risso* (*Cz2*) and *Citrus Aurantiifolia (Christm.) Swingle* (*Cz3*) epicarp by two extraction methods (maceration, soxhlet) and four solvents (hexane, dichloromethane, ethyl acetate, methanol). Data are means of 3 replicates ± standard deviation. Different letters above the bars represent a significant difference ($p < 0.05$) between treatments according to Student-Newman-Keuls test.

4. Conclusion

This study concluded that the best source of polyphenols among the three citrus species is in the following order for the edible part: *Citrus aurantifolia* > *Citrus limon* > *Citrus limetta*. On the other hand, for the non-edible part the order is as follows: *Citrus aurantifolia* > *Citrus limetta* > *Citrus limon*. The best source of flavonoids in both parts of the fruit is in the following order: *Citrus aurantifolia* > *Citrus limetta* > *Citrus limon*. The three species presented the exact amounts of condensed tannins for both parts of the fruit. Statistical analysis showed significant differences ($p<0.05$) for the content of polyphenols and total flavonoids in the three species studied. Regarding the extraction method, the antiradical activities of the analysed samples showed a significant difference ($p<0.05$). The results showed that the extracts obtained by Soxhlet had the highest antioxidant capacity compared to those of maceration, whatever the extraction solvent. Citrus epicarp and pulp of the three species studied showed intense antioxidant activity, making the fruit a viable source of natural antioxidants for the food industry. It is interesting to examine its use as a natural antioxidant ingredient in several finished food products.

5. Acknowledgment

The authors are very thankful to the Department of Biology, Faculty of Science, Ibn Tofail University, Kenitra, Morocco; University Cadi Ayyad, Marrakech, Morocco; National Institute of Agricultural Research, Marrakesh, Morocco and Department of Biology, Faculty of Sciences and Technologies-Gueliz, Cadi Ayyad University, Marrakesh, Morocco. All authors read and approved the final version of the manuscript.

6. References

[1] D. Mastorakou, M. Rabée, P. Salen, G. Pounis, M. de Lorgeril, Mediterranean diet: A health-protective dietary pattern for modern times, Analysis in Nutrition Research, Chapter 9, Book Elsevier Publisher, p.p. 233-258, 2019. https://doi.org/10.1016/C2017-0-01792-5

[2] C. T. Gallardo-Rivera, A. Lu, M. Z. Treviño-Garza1, E. García-Márquez, C. Amaya-Guerra, C. Aguilera, J. G. Báez-González, Valorization of almond (*Prunus serotina*) by obtaining bioactive compounds, Fron. Nut., 8 (2021) 663953. https://doi.org/10.3389/fnut.2021.663953

[3] Food and Agriculture Organizations (FAO) of the United Nations 2025. http://www.fao.org/faostat/en/#data.

[4] R. K. Saini, A. Ranjit, K. Sharma, P. Prasad, X. Shang, K. G. M. Gowda, Y. S. Keum, Bioactive compounds of *Citrus* Fruits: A review of composition and health benefits of carotenoids, flavonoids, limonoids, and terpenes, Antioxidants, 11 (2022) 239. https://doi.org/10.3390/antiox11020239

[5] X. Lu, C. Zhao, H. Shi, Y. Liao, F. Xu, H. Du, H. Xiao, J. Zheng, Nutrients and bioactives in *citrus* fruits: Different *citrus* varieties, fruit parts, and growth stages, Crit. Rev. Food Sci. Nutr., 63 (2021) 1–24. https://doi.org/10.1080/10408398.2021.1969891

[6] M. Addi, A. Elbouzidi, M. Abid, D. Tungmunnithum, A. Elamrani, C. Hano, An overview of bioactive flavonoids from *Citrus* fruits, Appl. Sci., 12 (2022) 1-29. https://doi.org/10.3390/app12010029

[7] B. Singh, J. P. Singh, A. Kaur, N. Singh, Phenolic composition, antioxidant potential and health benefits of *citrus* peel, Food. Res. Int., 132 (2020) 109-114. https://doi.org/10.1016/j.foodres.2020.109114

[8] A. Iqbal, P. Schulz, and S. S. Rizvi, Valorization of bioactive compounds in fruit pomace from agro-fruit industries: Present Insights and future challenges, Food. Biosci., 44 (2021) 101-384. https://doi.org/10.1016/j.fbio.2021.101384.

[9] R. Ashouri, S. A. H. Mirzahosseini, H. Shirkhanloo, A. Rashidi, N. Mansouri, Synthesis of carbon quantum dots from olive stones for efficient adsorption of benzene

from the ambient air, J. Nanostruct., 11 (2021) 480–497. https://doi.org/10.22052/JNS.2021.03.007

[10] H. B. Li, K. W. Cheng, C. C. Wong, K. W. Fan, F. Chen, Y. Jiang, Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae, Food Chem., 102 (2007) 771–776. https://doi.org/10.1016/j.foodchem.2006.06.022

[11] A. M. Shraim, T. A. Ahmed, Determination of total flavonoid content by aluminum chloride assay: A critical evaluation, LWT, 150 (2021) 111932. https://doi.org/10.1016/j.lwt.2021.111932

[12] A. E. Hagerman, Tannin Handbook, 2éme edition, Miami University, Oxford, USA, 116, 2002. https://cir.nii.ac.jp/crid/1570291225292849152

[13] İ. Gulcin, S. H. Alwasel, DPPH radical scavenging assay, Processes, 11 (2023) 2248. https://doi.org/10.3390/pr11082248

[14] L. Puigventós, M. Navarro, E. Alechaga, Determination of polyphenolic profiles by liquid chromatography-electrospray-tandem mass spectrometry for the authentication of fruit extracts, Anal. Bioanal. Chem., 407 (2015) 597–608. https://doi.org/10.1007/s00216-014-8298-2

[15] M. I. Ibrahim, A. E. Hegazy, Antioxydant activities of orange peel extract, Food. Sci. Tech., 18 (2012) 684–688. https://doi.org/10.5829/idosi.wasj.2012.18.05.64179

[16] D. Atmani, N. Chaher, M. Berboucha, K. Ayouni, H. Lounis, H. Boudaoud, N. Debbache, Antioxidant capacity and phenol content of selected Algerian medicinal plants, Food. Chem., 112 (2009) 303–309. https://doi.org/10.1016/j.foodchem.2008.05.077

[17] O. R. Alara, N. H. Abdurahman, Extraction of phenolic compounds: A review, Curr. Res. Food Sci., 4 (2021) 200-214. https://doi.org/10.1016/j.crfs.2021.03.011

[18] R. Murugan, T. Parimelazhagan, Comparative evaluation of different extraction methods for antioxidant and anti-inflammatory properties from *Osbeckia parvifolia Arn.* –An in vitro approach, J. King. Saud. Univ. Sci., 26 (2014) 267–275. https://doi.org/10.1016/j.jksus.2013.09.006

[19] R. Damaj, A. Sabbah, G. Nasser, M. B. Francis, A. Hijazi, H. Annan, A. A. A. AlRekaby, H. Rammal, Antioxidant activity and chemical composition of the ethanolic extract from leaves and stems of the Lebanese *Eryngium Creticum*, J. Multi. Eng. Sci. Tech., 3 (2016) 5813-5823. https://www.jmest.org/wp-content/uploads/JMESTN42351824.pdf

[20] S. Zeidan, A. Hijazi, H. Rammal, A. Kobaissi, B. Badran, Extraction of phenolic compounds and flavonoids from *eryngium creticum L.* by conventional and non-conventional extraction techniques, World J. Phar. Phar. Sci., 3 (2014) 1889–189. https://www.wjpps.com/

[21] S. Teimoori, A. H. Hassani, M. Panahi, N. Mansouri, An immobilization of aminopropyl trimethoxysilane-phenanthrene carbaldehyde on graphene oxide for toluene extraction and separation in water samples. Chemosphere, 316 (2023) 137–800. https://doi.org/10.1016/j.chemosphere.2023.137800

[22] Z. Mohammedi, F. Atik, Impact of solvant extraction type on total polyphenols content and biological activity from *Tamarixaphylla (L.).* karst, Int. J. Pharma. Bio. Sci., 2 (2011) 609–615. https://ijpbs.net/abstract.php?article=NTk1

[23] O. M. Andersen, K. R. Markham, Flavonoid: Chemistry, Biochemistry, and Applications, Taylor and Francis Group United States of America, 2005. https://doi.org/10.1201/9781420039443

[24] A. Ghasemzadeh, H. Jaafar, A. Rahmat, Effects of solvent type on phenolics and flavonoids content and antioxidant activities in two varieties of young ginger (*Zingiber officinale Roscoe*) extracts, J. Med. Plants Res., 5 (2011) 1147–1154.

https://academicjournals.org/article/article1380532263_Ghasemzadeh%20et%20al.pdf

[25] A. Barchan, M. Bakkali, A. Arakrak, R. Pagán, A. Laglaoui, The effects of solvents polarity on the phenolic contents and antioxidant activity of three *Mentha* species extracts, Int. J. Curr. Microbiol. App. Sci., 3 (2014) 399–412. http://www.ijcmas.com

[26] A. C. Mendes Hacke, J. A. Marques, J. C. Rebuglio Vellosa, A. A. Boligon, F. D. D'Avila da Silva, D. Souza, J. S. Bonini, J. B. Teixeira Rochad, R. Picada Pereira, Ethyl acetate fraction of *Cymbopogon citratus* as a potential source of antioxidant compounds, New J. Chem., 42 (2018) 3642–3652. https://doi.org/2018/NJ/C7NJ04352J

[27] F. Al juhaimi, C. Saglam, M. Musaozcan, Antioxidant properties and total phenolic content of two solvent extraction extracts of some plants belong to *Labiatae* family, Asian J. Chem., 25 (2013) 3011–3013. https://doi.org/10.14233/ajchem.2013.13482

[28] N. Khalfan Al-Musharfi, H. Suliman Al-Wahaibi, S. Alam Khan, Comparison of ascorbic acid, total phenolic content and antioxidant activities of fresh juices of six fruits grown in Oman, Food. Process. Tech., 6 (2015) 513. https://doi.org/10.4172/2157-7110.1000513

[29] M. Pallavi, C. K. Ramesh, V. Krishna, N. Sameera Parvee, L. Nanjunda Swamy, Quantitavie phytochemical analysis and antioxydant activities of some *citrus* fruits of South India, Asian J. Pharm. Clinical. Res., 10 (2017) 198–205. https://doi.org/10.22159/ajpcr.2017.v10i12.20912

[30] A. A. Damián-Reyna, J. C. González-Hernández, R. Maya-Yescas, C. J. Cortés-Penagos, M. C. Chávez-Parga, Polyphenolic content and bactericidal effect of Mexican *Citrus limetta* and *Citrus reticulatade*, J. Food. Sci. Technol., 54 (2017) 531–537. https://doi.org/10.1007/s13197-017-2498-7

[31] K. Ghasemi, Y. Ghasemi, M. A. Ebrahimzadeh, Antioxydant activity, phenol and flavonoide contents of 13 *citrus* species peels and tissues, Pak. J. Pharm. Sci., 22 (2009) 277-281. https://pubmed.ncbi.nlm.nih.gov/19553174/

[32] S. Teimoori, A. H. Hassani, New extraction of toluene from water samples based on nano-carbon structure before determination by gas chromatography, Int. J. Environ. Sci. Technol., 20 (2023) 6589–6608. https://doi.org/10.1007/s13762-023-04906-9

[33] R. Ashouri, N. Mansouri, Dynamic and static removal of benzene from air based on task-specific ionic liquid coated on MWCNTs by sorbent tube-headspace solid-phase extraction procedure, Int. J. Environ. Sci. Technol., 18 (2021) 2377-2390. https://doi.org/10.1007/s13762-020-02995-4

[34] A. Faghihi-Zarandi, H. Shirkhanloo, C. Jamshidzadeh, A new method for removal of hazardous toluene vapor from air based on ionic liquid-phase adsorbent, Int. J. Environ. Sci. Technol., 16 (2019) 2797-2808. https://doi.org/10.1007/s13762-018-1975-5

[35] A. Kumaran, R. Joel Karunakaran, *In Vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India, LWT-Food Sci. Technol., 40 (2007) 344-352. https://doi.org/10.1016/j.lwt.2005.09.011

[36] K. R. Narayana, M. S. Reddy, M. R. Chaluvadi, D. R. Krishna, Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential, Indian. J. pharm., 33 (2001) 2–16. https://journals.lww.com/iphr/pages/default.aspx

[37] C. Sánchez-Moreno, J. A. Larrauri, F. Saura-Calixto, A procedure to measure the antiradical efficiency of polyphenols, J. Sci. Food. Agric., 76 (1998) 270–276. https://doi.org/10.1002/(SICI)1097-0010(199802)76:2<270::AID-JSFA945>3.0.CO;2-9

[38] M. Arjomandi, H. Shirkhanloo, A review: Analytical methods for heavy metals

determination in environment and human samples, Anal. Methods Environ Chem. J., 2 (2019) 97–126. https://doi.org/10.24200/amecj.v2.i03.73

[39] L. Rubió, M. J. Motilva, M. P. Romero, Recent advances in biologically active compounds in herbs and spices: a review of the most effective antioxidant and anti-inflammatory active principles, Critical Rev. Food Sci. Nut., 53 (2013) 943–953. https://doi.org/10.1080/10408398.2011.574802

[40] M. M. Asl, H. Shirkhanloo, N. Mansouri, S. A. R. H. Seyed Mirzahosseini, F. Atabi, Functionalized graphene oxide with bismuth and titanium oxide nanoparticles for efficiently removing formaldehyde from the air by photocatalytic degradation–adsorption process, J. Anal. Test, 7 (2023) 444–458. https://doi.org/10.1007/s41664-023-00272-0

[41] A. Faghihi-Zarandi, J. Rakhtshah, B. B. Yarahmadi, A rapid removal of xylene vapor from environmental air based on bismuth oxide coupled to heterogeneous graphene/ graphene oxide by UV photo-catalectic degradation-adsorption procedure, J. Environ. Chem. Eng., 8 (2020) 104–193. https://doi.org/10.1016/j.jece.2020.104193

[42] S.Teimoori,A. H. Hassani, M. Panahi, N. Mansouri, Rapid extraction of BTEX in water and milk samples based on functionalized multi-walled carbon nanotubes by dispersive homogenized-micro-solid phase extraction, Food Chem., 421 (2023) 136–229. https://doi.org/10.1016/j.foodchem.2023.136229

[43] M. Mohammadi Asl, N. Mansouri, S.A.R Haji Seyed Mirzahosseini, F. Atabi, Simultaneity comparative evaluation of toluene removal from the air by adsorption and UV semi-degradation-based adsorption procedure, Int. J. Environ. Sci. Technol., 21 (2024) 6677–6694. https://doi.org/10.1007/s13762-024-05503-0