Article

Phytochemical composition and antioxidant activity in three basil varieties due to the effect of different solvents

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Abstract

Basil (*Ocimum basilicum*) is a very important crop in the world and in Mexico for the well-known specialties of Mediterranean cuisine. There is a growing demand for basil in the United States of North America and Europe due to its antioxidant content. Nowadays, to change synthetic antioxidants for natural ones is a trend in the food industry. Interest in analyzing natural, non-toxic and healthy products that work as antioxidants has increased. Basil contains high levels of secondary metabolites. With the aim of determining the extraction potential of different solvents (hexane, methanol, petroleum ether and ethanol) in three varieties of basil (Lemon, Cinnamon and Red Rubin). In the present study, quantification analyses were carried out for total phenolic compounds, with values between 0.5 and 17.9 mg based on gallic acid per gram of sample in dry weight, total flavonoids obtaining values that ranged between 2.4 and 10.8 mg of quercetin per gram of sample in dry weight and determination of antioxidant activity with results between 57.4-409.4 μmol Trolox per gram of sample in dry weight of the three varieties of basil (Lemon, Cinnamon and Red Rubin) and the different solvents as extraction media (methanol, hexane, petroleum ether and ethanol). Results of greater efficiency were obtained for the different variables measured when the solvent used was methanol in the Lemon and Cinnamon varieties and ethanol in the Red Rubin variety, without finding a significant difference to the extraction with methanol.

Keywords: extraction, flavonoids, phenolic compounds, secondary metabolites.

Reception date: January 2022
Acceptance date: March 2022

Introduction

Basil (*Ocimum basilicum* L.) is an aromatic plant used directly as a spice, medicine, food, ornamental plant and as a raw material for different industries. Basil is the most cultivated due to its high economic value, popularity and demand for the production of essential oils in many continents of the world for its numerous economic, medicinal and aromatic values (Egata, 2021). Currently, the search for new natural antioxidants has intensified, in particular in the cultivated plant species (Thakur and Vasudevan, 2019).

Plants have been an inexhaustible source of medicines and recently, much emphasis has been placed on finding new therapeutic agents based on medicinal plants (Barbouchi *et al*., 2020). When talking about the phytochemical composition and concentration in cultivated plants, they are greatly affected by some factors, such as genetics, environmental growing conditions, harvesting and post-harvest treatment.

The extraction of metabolites using a different method and the type of solvent used also play an essential role in the level of metabolites extracted (Rafi *et al*., 2020). The consistency in the composition and concentration level of the bioactive compounds will give a different biological activity for the different samples studied, which can be rich in phenolic compounds such as phenolic acid, flavonoids and tannins and those compounds are known for their potency as antioxidants (Pereira *et al*., 2019).

Plants are rich sources of biochemical compounds such as phenols, fatty acids, saponins, essential oils or alkaloids, which have proven therapeutic properties but are less studied and valued (Fajemiroye *et al*., 2016). *Ocimum* species are plants that have also not been valued in the past, but there is an increase in interest in these plant species in recent years (Rezzoug *et al*., 2019; Egan *et al*., 2020). The species *Ocimum* is also characterized by an abundance of compounds such as phenolic acids and volatile oils (Antonescu *et al*., 2021).

The antioxidant compounds in plant extracts have different molecular structures and polarities. Based on their molecular structure, antioxidant compounds with their chemical characteristics and polarities may or may not be soluble in a specific solvent (Rafi *et al*., 2020). Polar solvents are frequently used for the extraction of polyphenols from plant matrices (Altemimi *et al*., 2017). Normally, the recovery of antioxidant compounds is combined with different extraction methods, depending on their chemistry and distribution in the matrix (Ameer *et al*., 2017). For the isolation of polyphenols and other compounds from plant sources, many different approaches have been applied.

Among them, solvent extraction remains the most widely used extraction procedure, mainly due to its low processing cost, ease of use and wide range of applicability (Brglez *et al*., 2016). The selection of solvent is critical and often determinant in the extraction yields and subsequent antioxidant activities of plant extracts, due to the influence of the solvent on the solubilization of antioxidant compounds with different chemical structures and polarity (Do *et al*., 2014).

For the recovery of polyphenols, polar solvents are often used, especially aqueous mixtures that contain methanol, ethanol, acetone or ethyl acetate (Monteiro *et al*., 2020). Methanol and ethanol have been widely used to extract antioxidant compounds from various plants, including plants traditionally used for medicinal purposes (Do *et al*., 2014; Aboshora *et al*., 2015; Dhawan and Gupta 2016). Therefore, the objective of this study was to determine the solvent with the greatest potential for extraction of antioxidant compounds present in three varieties of basil.

Materials and methods

Location of the experiment

The basil cultivars were established in March 2020 in a greenhouse with sliding plastic covers and anti-aphid mesh with semi-automatic control, located at the Polytechnic University of Gómez Palacio, Durango, 25° 38’ 19.83” north latitude, 103° 31’ 52.12” west longitude. The temperature and relative humidity values of the greenhouse were kept between 25 and 30 °C and 70-80%, respectively.

Establishment of cultivars

Three basil cultivars were planted, their seeds were from donations from organic basil plantations in Baja California Sur (Lemon, Cinnamon and Red Rubin). Sowing was carried out in polystyrene germination trays of 250 cavities and peat moss was used as a substrate. The trays were watered twice a day with water until the day of transplantation. When the seedlings had three to four true leaves and a height of 10 to 15 cm, they were transplanted into black plastic pots of one gallon capacity, which contained a mixture of sand:perlite (80:20).

Preparation of samples and obtaining extracts

At 35 days after transplantation, leaf samples of the three basil varieties were taken until samples of all the plants in the experiment were obtained. All samples were washed with distilled water and then the excess water was removed on kraft paper. The drying process was carried out at room temperature (25 ±2 °C) for 15 days. After the dehydration time, the samples went through a grinding process until obtaining a powder. This process was carried out in a blender (Hamilton Beach).

After that, the pulverized samples were stored at 5 °C for the subsequent obtaining of extracts (Abkhoo and Jhani, 2016). Extracts were obtained by the solid-liquid extraction technique by adding 1 g of each of the pulverized samples placed in test tubes. Ten milliliters of each of the solvents used (hexane, methanol, petroleum ether, Jalmek® ethanol) were added. An automated stirrer (Stuart®) was used for 24 h to keep the mixture under stirring at room temperature (25 ±2 °C).

After the time established in stirring, the samples were left to stand in a rack for the phase change and precipitation of each sample. When the separation was observed, the extract was obtained, leaving the precipitated sample in the tubes. The extracts were concentrated to obtain the compounds of interest with a rotary evaporator (Buchí, model-210) and a water bath (RIOSA, Mexico) at 35-40 °C. The extracts obtained were stored in deep freezing at -20 °C until their analysis (Ramírez *et al*., 2019).

Quantification of total phenolics (TPCs)

The determination of total phenolic compounds was performed by spectrophotometry (Genesys 10 UV equipment USA), based on the oxide-reduction colorimetric reaction according to Zamora (2016) with some modifications. To 30 µl of extract, 2 ml of distilled water, 250 µl of Folin-Ciocalteu reagent (analytical grade, Sigma-Aldrich, St. Louis MO, USA) were added, then 1 ml of Na_2CO_3 at 10% w/v was added, and it was measured to 5 ml with distilled water. The samples were read at a wavelength of 765 nm after staying 1 h at room temperature and in the absence of light. For the calculation of total phenolics, a calibration curve was performed with analytical grade gallic acid (Sigma-Aldrich St. Louis MO, USA). The phenolic content was expressed in mg GAE g^{-1} of DW (mg of gallic acid equivalents per gram of dry weight).

Quantification of flavonoids (TFVs)

The flavonoids were quantified by a spectrophotometric analysis (Genesys 10 UV equipment USA) based on the formation of a complex between the Al (III) ions and the carbonyl and hydroxyl groups of the flavonoid, as described by Zamora (2016) with some modifications. Fifty microliters of extract were mixed with 100 µl of $AlCl_3$ at 5% w/v in ethanol, 100 µl of 1M sodium acetate, and they were measured to 5 ml with analytical grade methanol (JT Baker). The flavonoid compounds were measured at a wavelength of 425 nm after 30 minutes of staying in the absence of light. Prior to the reading, a calibration curve was made with analytical grade quercetin (Sigma-Aldrich St. Louis MO, USA). The flavonoid content is expressed in mg QE g^{-1} of DW (mg of quercetin equivalents per gram of dry weight).

Determination of antioxidant capacity (AA)

Antioxidant capacity was measured according to the methodology proposed by Chaves *et al*. (2016) with modifications. The ABTS radical (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) was obtained by the reaction of ABTS (7 mM) with potassium persulfate ($K_2S_2O_8$) 2.45 mM, then measured with water at a volume of 10 ml and incubated at room temperature (±25 °C) and in the dark for 18 h. The ABTS radical was diluted in ethanol until obtaining an absorbance value between 0.7 (±0.1) at a wavelength of 754 nm.

The extracts were dissolved in ethanol at a concentration of 200 mg L^{-1}. After that, 50 µl of sample and 1 950 µl of ABTS were placed in a test tube. They were stirred for 1 min and kept in the dark for 10 min. After the reaction, the absorbance was read at 754 nm (Genesys 10 UV spectrophotometer USA). The results are expressed as a percentage of inhibition in micromoles Trolox per each gram of sample in dry weight (µmol Trolox g^{-1} DW).

Statistical analysis

The statistical analysis was carried out by means of an analysis of variance using the Statistical Release Software 7.0 and the comparison of means was performed using the Tukey test with a significance value of $p \leq 0.05$. A 3 x 4 factorial arrangement was used in a completely randomized design, where the factors were: genotype and extraction solvent. Three repetitions were performed per treatment.

Results and discussion

The present study showed differences for the factors studied ($p \leq 0.05$), which can be seen in Table 1. For the factor genotype, the three basil varieties showed similar results in terms of the variables analyzed, obtaining ranges between 7.18-6.57 and 6.6-6.05 for TPCs and TFVs, respectively. In the variable AA, a difference is observed between the genotypes, with Cinammon being the variety with the highest AA, followed by Lemon and finally Red Rubin (12% and 17% higher, respectively).

Table 1. Average TFVs, TPCs and AA values of three basil varieties and four extraction solvents.

Characteristics	TFVs (mg QE g^{-1} DW)	TPCs (mg GAE g^{-1} DW)	AA (μmol Trolox g^{-1} DW)
		Variety	
Lemon	6.6 ±0.88 a	7.18 ±0.85 a	220.79 ±32.08 b
Cinnamon	6.25 ±0.77 a	6.57 ±1.86 a	249.5 ±36.38 a
Red Rubin	6.05 ±1.05 a	6.8 ±2.07 a	207.22 ±38.12 b
		Extraction solvents	
Hexane	3.48 ±0.19 c	7.62 ±0.68 b	61.79 ±3.3 c
Methanol	10.64 ±0.13 a	16.82 ±0.34 a	309.22 ±34.29 a
Petroleum ether	7.26 ±0.27 b	0.72 ±0.18 d	215.06 ±10.13 b
Ethanol	3.81 ±0.46 c	2.24 ±0.37 c	317.28 ±23.47 a
		Interaction	
Variety∗solvent	**	**	**

TFVs= total flavonoids; TPCs= total phenolic compounds; AA= antioxidant activity; **= highly significant. Different letters in column indicate a highly significant difference at $p \leq 0.05$.

For the factor extraction solvent, it can be observed that, for the variables TFVs and TPCs, the best solvent is methanol with a difference in the percentage of extraction of 68% for flavonoid extraction compared to the hexane solvent and 95% of efficiency for the extraction of phenolic compounds compared to the petroleum ether solvent. Regarding the variable AA, in the factor extraction solvent, it was obtained that the most efficient was ethanol but without significant difference compared to the methanol solvent.

Total flavonoids

The results of the TFV content are shown in Figure 1. The flavonoid content in this experiment ranged from 2.4 to 10.8 mg QE g^{-1} DW, obtaining that the solvent with the highest efficiency for the extraction of these compounds was methanol for the three basil cultivars used, followed by the petroleum ether solvent and with the ethanol and hexane solvents registering the lowest extraction percentages (38 and 22%, respectively) with respect to the highest value of TFVs. This study coincides with the results obtained by Do *et al*. (2014), who reported that the extraction of flavonoids from plants was strongly influenced by the different solvents used.

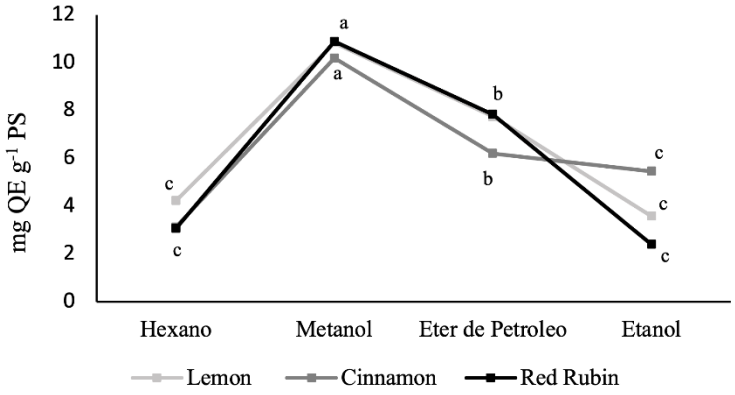

Figure 1. Concentration of total flavonoids in extracts of three basil varieties obtained by four different solvents. The literals indicate the significant difference between the different extraction solvents for each variety of basil studied.

The content of TFVs in aqueous extracts of the flower of *M. acuminata* (159 45 mg QE 100 g^{-1}) and *P. tetragonolobus* (172 44 mg QE 100 g^{-1}) was consistent with that reported by Ng and See (2019) (196 3 mg QE 100 g^{-1}) and Gan *et al*. (2017) (185 mg QE 100 g^{-1}). Compared to water extracts, methanol significantly increased the TFV yield of the flower of *M. acuminata* (24%) and *P. tetragonolobus* (73%), while n-hexane provided the lowest TFV content in both plants. The above results are similar to those obtained in this experiment, where the solvent with the highest extraction capacity was methanol and that with the lowest efficiency was hexane.

Flavonoids are the most abundant polyphenols with several reactive phenolic hydroxyl groups in benzene rings (Altemimi *et al*., 2017), but reference is also made to the fact that the differences obtained in the different extractions by each solvent could be attributed to the variation in the biochemical composition of the plant, where the phenolics, in particular flavonoids, are the main extractable phytochemicals (Joseph *et al*., 2014). Regarding the solvent with the highest extraction capacity (methanol), it is known that in tannin-protein complexes, most flavonoids exist in glycosides and methanol favors the extraction of these polar complexes of sugar-flavonoids (Ng *et al*., 2020).

Total phenolic compounds

Phenolic compounds are ubiquitous in most medicinal plants and constitute an essential part of the human diet due to their antioxidant properties and many other beneficial health properties (Balasundram *et al*., 2006). TPCs range between the values of 0.2 to 17.9 mg GAE g^{-1} DW, showing the wide range of extraction between the different solvents used for the three varieties of basil analyzed. The following figure (Figure 2) shows the extraction efficiency of each solvent used for each variety of basil analyzed.

The methanol solvent was the most efficient extraction agent for TPCs; extraction values with this solvent ranged from 15.8, 16.7 and 17.9 mg GAE g^{-1} DW for Lemon, Cinnamon and Red Rubin varieties, respectively, followed by hexane, ethanol and petroleum ether. According to the chemical structure of hexane, it is known that it is a solvent with lower polarity than petroleum ether (Nawaz

et al., 2020), which explains why it worked more efficiently in the extraction of TPCs than in the extraction of TFVs, since, according to the classification of phenolic compounds, flavonoids are a subgroup of phenols that do not always have affinity with low polar solvents (Nawaz *et al*., 2020).

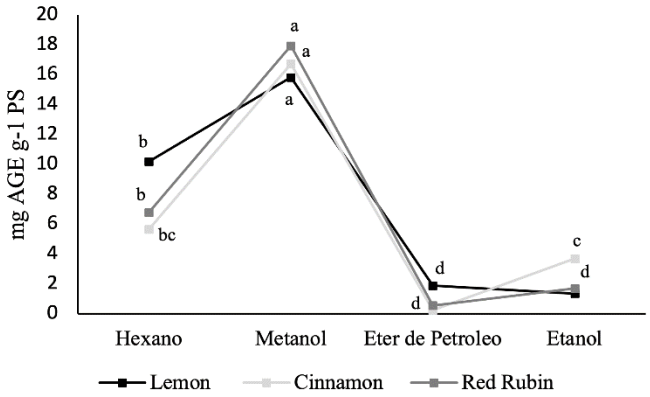

Figure 2. Concentration of total phenolic compounds in extracts of three basil varieties obtained by four different solvents. The literals indicate the significant difference between the different extraction solvents for each variety of basil studied.

Authors such as Orlando *et al*. (2019) indicated that the extraction yields of polyphenols varied considerably between the different plant varieties. Salih *et al*. (2020) did not obtain a good extraction of polyphenols from macerated or cooked samples when using water as an extractant, compared to the use of methanol and ethanol. However, the use of other solvents leads to optimal extraction of bioactive compounds (Lezoul *et al*. 2020).

In this sense, the polarity of methanol is greater than that of ethanol; for this reason, very polar phenolic acids, such as cinnamic or benzoic acids, could be extracted more easily, increasing the content of total polyphenols in the extracts obtained (Stalikas, 2007). This explains the results of the present study, since, when methanol was used, the extraction of TPCs was greater compared to the rest of the solvents used in the different varieties of basil.

Antioxidant activity

In the present study, free radical scavenging properties were shown, and the values obtained showed indicators of antioxidant capacities based on the elimination of the free radical used in the ABTS technique. It was observed that the values for the free radical scavenging activity are higher when the solvent used was methanol for the Lemon and Cinnamon varieties, it was not the same case for the Red Rubin variety since, in these extracts, the greatest efficiency of antioxidant activity occurred when the solvent used was ethanol (Figure 3).

Table 1 mentions that the variety of basil with greater antioxidant activity, according to the averages yielded in the statistical analysis, is Cinnamon (249.5 μmol Trolox μg^{-1} DW); however, when analyzing the factors in the interaction, it was observed that the AA is higher in the Red Rubin basil variety when the solvent used is ethanol with a value of 409.4 μmol Trolox μg^{-1} DW. It should be noted that the Red Rubin basil variety has different characteristics in relation to the other two varieties, such as its pigmentation, and the quantity of secondary metabolites it has (Ramírez *et al*., 2019).

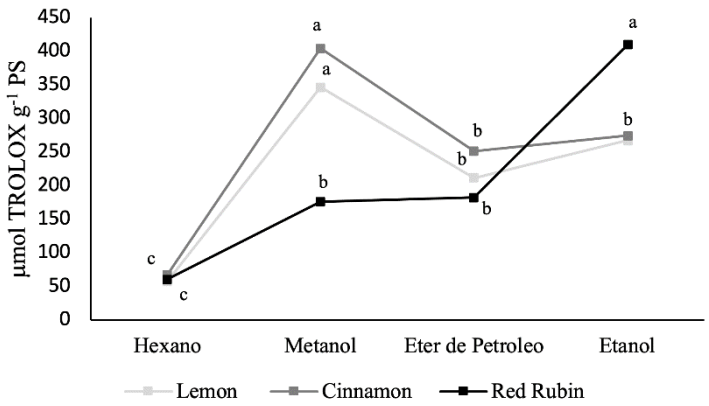

Figure 3. Micromolar concentration of antioxidant activity by ABTS technique in extracts obtained by different solvents for three basil varieties. The literals indicate the significant difference between the different extraction solvents for each variety of basil studied.

In the study conducted by Dowlath *et al.* (2020), it was reported that they found no significant differences between the extraction solvents petroleum ether and chloroform when they analyzed the antioxidant compounds (TPCs and AA) of *Cardiospermum halicacabum*; however, these solvents had a significant difference with respect to the ethanol solvent in the measurement of antioxidant capacity by the DPPH method. The above findings coincide with those obtained in this experiment since the solvent with the highest efficiency for free radical scavenging was ethanol and methanol (Dowlath *et al*., 2020).

Because the solvent used for extraction shows variations in the radical scavenging capacity of the extracts (Dhanani *et al*., 2017), it demonstrates the influence of the solvent on the antioxidant activity of the crude extracts (Bhebhe *et al*., 2016). This is supported by studies on *Macadamia tetraphylla* shells, herbal infusions of *Limnophila aromatic* and *Withnaia somnifera* (L.), where there is a strong influence of the solvent on the antioxidant activity of plant extracts (Do *et al*., 2014; Dailey and Vuong, 2015). This variation in antioxidant activity may possibly be associated with the compounds of the plant, such as phenolic constituents, which are soluble based on the polarity of the solvents used for extraction or other secondary metabolites present in each of the varieties studied (Dowlath *et al*., 2020).

Conclusions

This study showed that solvents with different polarities have variable effects on the extraction of phytochemical compounds and antioxidant activity of different basil varieties. The Lemon and Cinnamon basil varieties showed higher content of phytochemical compounds and antioxidant activity in extracts with the methanol solvent, while ethanol had the same extraction efficiency as methanol for the Red Rubin variety. The results obtained in this study could serve as a classification tool to identify the ideal extraction solvents for different medicinal plants.

Considering the wide application of the solvent-based extraction system in the food and manufacturing industries, further research on the phytochemical profiles of different plant solvent extracts through high-resolution liquid chromatography-mass spectrometry is essential for the isolation and characterization of the secondary metabolites of the plant and their study with different solubilities.

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