Phenolic Compounds Extraction from *Achyrocline satureioides, Curcuma longa, Foeniculum vulgare* and *Rosmarinus officinalis.*

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There is an increasing interest in the population in the consumption of consume natural functional foods since it can result in benefits for human health. One attractive class of bioactive compounds is some phenolic substances. Some condiments, aromatic plants, and natural dyes used daily in the preparation of food have these compounds. Some examples are turmeric (*Curcuma longa* L.), macela (*Achyrocline satureioides*), fennel (*Foeniculum vulgare*), and rosemary (*Rosmarinus officinalis*). These compounds have confirmed pharmacological anticancer and anti-inflammatory activities or are used as food preservatives. The bioactive compounds can be present in different parts of the plants such as in turmeric tubers, macela flowers, fennel seeds, and rosemary leaves. The main goal of this paper was to maximize the yield of phenolic compounds in the extracts obtained by supercritical fluid extraction (SFE), hydrodistillation (HD), and low pressure solvent extraction (LPSE). The extracts were characterized by gas chromatography (GC) and by thin layer chromatography (TLC). The differences in the matrix (tuber, flower, seeds, and leaves) structures were observed by optical microscopy. The antioxidant activities of the extracts were determined. The largest extraction yield was obtained by LPSE for all tested plants, followed by SFE and HD. The LPSE yields were 9, 10, 13, and 14 %, for rosemary, fennel, macela and turmeric, respectively. The antioxidant activity was larger for the SFE extracts of rosemary, macela, and turmeric followed by the fennel essential oil (the HD fennel extract). It was possible to observe the structures in the different tested tissues.

INTRODUCTION

The presence of phenolic compounds in medicinal plants are responsible for the antioxidant and anti-inflammatory activities of these species, allowing them to be used as potential chemopreventives [1]. Several researchers have studied the use of phenolic compounds as therapeutic compounds with antioxidant activities [2] and several works suggest the use of a diet rich in phytochemicals to prevent and treat cardiovascular diseases and cancer [3], [4], [5], [6], [7]. Among the studied flavonoids (aglicones) the quercetin, myricetin, kaempferol present antioxidant activity. The gallic and rosmarinic acids were the most potent antioxidants among the simple phenolic acids [2].

Several vegetables and fruits have phenolic compounds with certified antioxidant activity such as spinach, broccolis, onion, eggplant, squash [8], [9], [5]. Some vegetable species used as source of bioactive compounds are found in the Brazilian regular diet such as turmeric (*Curcuma longa* L.), macela (*Achyrocline satureioides*), fennel (*Foeniculum vulgare*), and rosemary (*Rosmarinus officinalis*). These vegetables have scientifically proved functional properties [10], [11], [12], [13].

The phenolic compounds can be presented in special glandules into the vegetal structure, depending on the plant organ, the cultivation edafoclimatic conditions, stress, and age of the plant [14]. It is possible to find different contents of phenolic substances in the different organ or secretorial structure of the plant. The quercetin concentration changes even with position at the same leave surface, as was reported for *Nicotiana attenuata* [14]; the phenolic compounds were found mainly in the leaves and roots of *Centella asiatica*, but at lower concentration at the petioles. There is a strong correlation between the presence of phenolic compounds and the antioxidant activity of some extracts [15]. This correlation was observed for vegetables, apple and pineapple juices [15], [16], fruits, vegetables and grains [8].

Several methods are used to extract the phenolic compounds but, in order to keep the original characteristics, it is important to use methods that do not degrade them. Among the extraction methods, the supercritical fluid extraction has been satisfactorily used to obtain natural products extracts [13], [17], [18].

Thus, the main objectives of this work were: (i) compare the phenolic compounds yields in the extracts of turmeric (*Curcuma longa* L.), macela (*Achyrocline satureioides*), fennel (*Foeniculum vulgare*), and rosemary (*Rosmarinus officinalis*) obtained using supercritical carbon dioxide, hydrodistillation, and low pressure solvent extraction; (*ii*) determine the antioxidant activity of the extracts, and (*iii*) identify the phenolic compounds secretory structures in different types of matrices (tuber, flower, seeds, and leaves) by fluorescence microscopy.

I - MATERIALS AND METHODS

Raw material characterization - The material was comminuted in a knife mill and the particle size distribution of the solid was determined using an agitator containing sieves of the Tyler series meshes 24, 32 and 48, for the turmeric, fennel and rosemary; for macela it was impossible to determine the size distribution of the comminuted flowers and petals.

Microscopy procedure - The secretor structures were observed by fluorescence microscopy (Nikon, model Eclipse E2000, New York, USA); free hand-sectioned materials were treated with 2% of 2-aminoethyl diphenylborinate (methanol) using a microscopy with fluorescence illumination. The analyses were done at the Colloids and Bioreactions Engineering Laboratory, College of Chemical Engineering, Unicamp, Campinas, Brazil.

Extraction Methodologies - The extracts were obtained by Supercritical fluid extraction (SFE), low pressure solvent extract (LPSE) and hydrodistillation (HD). The SFE assays were done using a unit containing an extraction cell of approximately 221×10^{-6} m^3 (length of 37.5×10^{-2} m and internal diameter of 2.74×10^{-2} m) similar to the apparatus described by Braga et al [17]. The global yields, that is, the total amount of extractable solute were calculated as amount of extract obtained at the end of the extraction process, including the extracts recovered from the system tubes and the extracts obtained during the depressurization step [18]. The Low Pressure Solvent extracts (LPSE) were obtained in a fixed bed extractor (glass column of length of 20×10^{-2} m and internal diameter of 2.8×10^{-2} m). The solvent flow was controlled by a Masterflex Pump Controller (Cole Parmer Instrument Co, Chicago, USA); ethanol 96% (Merck, São Paulo, Brazil) was used, and the employed solid to solvent ratio was 1:10. The system was kept under operation for 2 h. The ethanol was removed from the extract using a rotavap (Laborota, model 4001, Viertrieb, Germany) with vacuum control (Heidolph Instruments GMBH, model Rotavac control, Viertrieb, Germany). The

volatile oil (essential) was obtained by hydrodistillation for 3h and recovered using ethyl ether. The separation of the solvent and extract was made under air flow.

Characterization of the extract - The chemical composition of the extracts were determined by GC; the Kovats program was used to identify the main compounds [21], [13], [22].Antioxidant Activity was determined using the coupled oxidation of linolenic acid and β-carotene: methodology of Hammerschmidt and Pratt (1978) with modifications for the SFE extracts as presented by Leal et al [10]. The quantification of phenolics contents was made using the Singleton and Rossi Method with the modifications of Cheung et al [19]. The TLC was carried out using the Wagner et al [20] methodology for flavonoids using 2-aminoethyl diphenylborinate as spray reagent.

II - RESULTS AND DISCUSSION

The highest extract yield for all the tested plants was obtained by LPSE followed by SFE and HD. The TLC analysis of the ethanol LPSE extract showed the presence of several undesired compounds. In general, the SFE yield was lower than the LPSE but the obtained extract was more pure and without traces of solvent.

For macela, turmeric and rosemary the SFE resulted in extracts with highest yields in phenolic compounds (Table 1) while the LPSE allowed the highest yield for fennel extracts. For macela extracts, the phenolic compounds content at the HD extract was approximately 50% lower than that of the LPSE and SFE extracts. The phenolic compounds concentration for the turmeric extracts was similar for LPSE and HD. The phenolic content in the fennel HD extract was 85% lower than the LPSE one and for rosemary the HD extract presents 97% lower content of phenolic compounds than that of the LPSE extract.

The highest phenolic compounds concentration was observed in the SFE turmeric extracts (26 mg/g).

Table 1. Total yield and phenolics content quantification of macela, turmeric, fennel and rosemary extracts

Specie	Extraction	Yield (%) (d.b.)	mg phenols/g extract ± amplitude × 10^{-3}
Macela	HD	0.12 ± 0.04	6 ± 3
	LPSE	12.6 ± 0.3	13.2 ± 0.05
	SFE	6.4 ± 0.4	14 ± 0.3
Turmeric	HD	3.1	7.1 ± 0.4
	LPSE	14	6.7 ± 0.1
	SFE	12	26 ± 2
Fennel	HD	3.0	2.4 ± 0.6
	LPSE	9.2	16.4 ± 0.5
	SFE	6.9	11 ± 1
Rosemary	HD	2.3	0.4 ± 0.03
	LPSE	9.5	8 ± 1
	SFE	4.6	12 ± 1

Figure 1 shows that SFE resulted in extracts with high antioxidant activity (80-100 %) for rosemary, macela, and turmeric. Even after 3 h the antioxidant activity was kept approximately constant for the SFE extracts. The LPSE turmeric extract presented high antioxidant activity for the tested period. The macela and rosemary LPSE extracts presented a decrease of the antioxidant activity from 70-80% after 1 hour of reaction and a decrease of 50-70% after three hours of reaction.

The antioxidant activity of the LPSE fennel extract was higher than the SFE one, but it was lower than for the extracts of the other tested plants. For the HD extracts, the antioxidant activity after 1 hour was comprehended between 40-60% decreasing to 30-50% after 3 hours of reaction. The fennel extract was the HD extract that presented the highest antioxidant activity. The LPSE extracts presented the largest number of compounds in both TLC and GC chromatograms. The most important compounds were obtained mainly by SFE and LPSE and the volatile compounds were obtained mainly by HD.

Figure 2 shows the transversal sections of fennel seeds, rosemary leaves, macela flowers and turmeric roots. The fluorescence indicates the presence of flavonoids at all analyzed fragments: in fennel seeds the cell layer closer to the surface showed higher yellow fluorescence intensity and the inner cells presented a greenish fluorescence; for rosemary leaves and macela petals it is possible to observe a lighter yellow fluorescence at all the surface; for the turmeric fragment it was possible to observe the presence of green, yellow, and orange fluorescence, indicating the possible presence of several classes of flavonoids.

CONCLUSION

The extracts of fennel seeds, rosemary leaves, macela flowers, and turmeric roots obtained by supercritical fluid extraction presented higher yields in phenolic compounds if compared with the ones obtained by LPSE. The phytochemical profile was a function of the extraction methodology (SFE, LPSE, and HD). The largest antioxidant activities of macela, turmeric, and rosemary were observed for the SFE extract while for fennel larger antioxidant activity was observed for the HD extract. Microscopy allowed observing the presence of flavonoids at different cells in fennel and turmeric and at all of the surface of rosemary leaves and macela petals.

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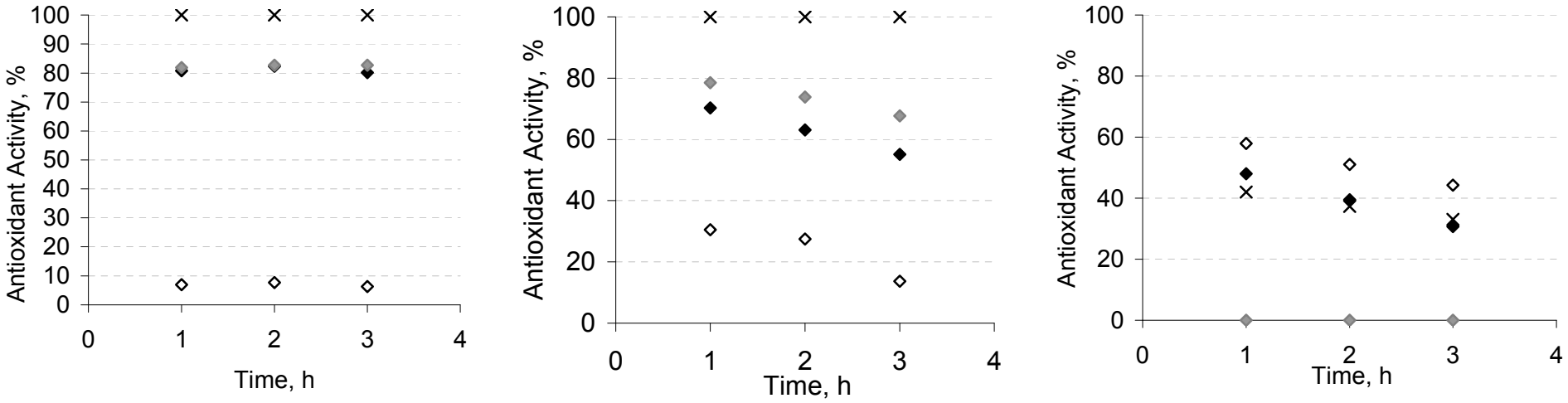

Figure 1. Antioxidant activity of extracts of fennel seeds, rosemary leaves, macela flowers and turmeric roots obtained by SFE, LPSE and HD extractions ○ Fennel ● Rosemary ● Macela × Turmeric

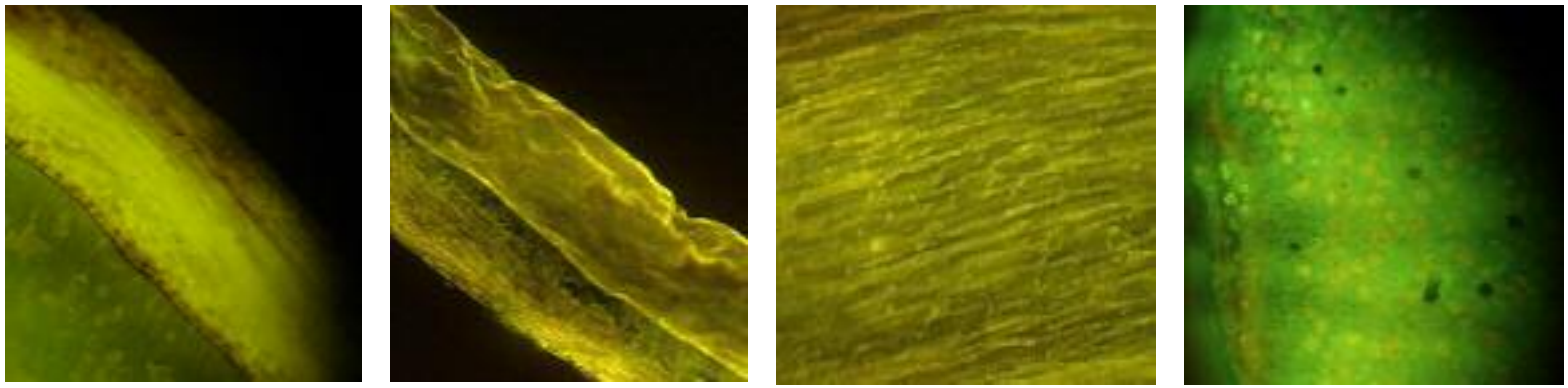

Figure 2. Fluorescence microscopy of hand-sectioned fennel seeds, rosemary leaves, macela flowers and turmeric roots (40×) using 2% of 2-aminoethyl diphenylborinate to identification of flavonoids.

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