

Optimizing preparation of the fennel (*foeniculum vulgare* L.) extracts by using ultrasound - methodology in accordance with the principles of green chemistry

Otimização da preparação dos extratos de funcho (*foeniculum vulgare* L.) através do uso de ultrassom – metodologia baseada nos princípios da química verde

Optimización de la preparación de los extractos de hinojo (*foeniculum vulgare* L.) mediante el uso de ultrasonidos - metodología de acuerdo con los principios de la química verde

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Abstract

Fennel (*foeniculum vulgare* L.) is a vegetal species that presents bioactive monoterpenes. These compounds are lipophilic substances almost insoluble in water. The best conditions to extract these substances, employs organic solvents or heating process. With the aim to developing new extractive methods in which the green chemical principles can be applied, this work was performed. The effect of the use of ultrasound during the preparation of fennel extracts by water infusion and by ethyl acetate maceration methods was verified. The ethyl acetate extracts, and the organic portion of the aqueous extracts were analyzed by gas chromatography coupled to mass spectrometry. The ethyl acetate extraction, without the use of ultrasound leads only to the extraction of two metabolites (fenchone and anethole), the maceration performed by using ultrasound by 5 and 10 minutes, promoted the extraction of seven metabolites (α -pinene, L-fenchone, estragole, anethole, β -caryophyllene, α -cadenol, and geranylgeraniol), while the use of ultrasound in times upper than 10 minutes leads to extracts with 4 metabolites (α -pinene, L-fenchone, estragole and limonene). The aqueous extracts submitted or not to the use of ultrasound led to the extraction of the same and five metabolites (D-limonene, L-fenchone, estragole, and anethole).

Keywords: Gas chromatography; Infusions; Teas; Fennel; *Foeniculum Vulgare* Mil.

Resumo

O funcho (*foeniculum vulgare* L.) é uma espécie vegetal que apresenta monoterpenos bioativos. Devido ao seu caráter lipofílico, tais substâncias são quase insolúveis na água, deste modo, os processos extrativos destes compostos, geralmente empregam solventes orgânicos ou processo de aquecimento. Com o objetivo de desenvolver novos métodos extrativos em que os princípios da química verde sejam aplicados, este trabalho foi realizado. Foi verificado o efeito da utilização de ultrassom na preparação de extratos de funcho por infusão em água e métodos de maceração em acetato de etila. Os extratos em acetato de etila e a porção orgânica dos extratos aquosos foram analisados por cromatografia gasosa acoplada à espectrometria de massa. A extração, por maceração em acetato de etila, sem o uso

de ultrassom, conduziu a extratos com apenas dois metabolitos (fenchona e anetol), a maceração realizada com auxílio de ultrassom por 5 e 10 minutos, promoveu a extração de sete metabolitos (α -pineno, L-fenchona, estragol, anetol, β -cariofileno, α -cadinol, e geranilgeraniol), enquanto a utilização de ultrassom em tempos superiores a 10 minutos leva a extratos com 4 metabolitos (α -pineno, L-fenchone, estragol e D-limoneno). Os extratos aquosos submetidos ou não ao uso do ultrassom conduziram a extração dos mesmos cinco metabolitos (D-limoneno, L-fenchona, estragol e anetol).

Palavras-chave: Cromatografia gasosa; Infusões; Chás; Funcho; *Foeniculum Vulgare Mil.*

Resumen

El hinojo (*foeniculum vulgare* L) es una especie vegetal que presenta monoterpenos bioactivos, que son sustancias lipofílicas casi insolubles en agua. Las mejores condiciones para extraer estas sustancias apolares de las especies vegetales, suelen emplear disolventes orgánicos o procesos de calentamiento. Con el fin de desarrollar nuevos métodos extractivos en los que se apliquen los principios de la química verde, se llevó a cabo este trabajo. Se comprobó el efecto del uso de ultrasonidos en la preparación de extractos de hinojo por los métodos de infusión en agua y maceración en acetato de etilo. Los extractos de acetato de etilo y la parte orgánica de los extractos acuosos se analizaron mediante cromatografía de gases acoplada a espectrometría de masas. La extracción en acetato de etilo, sin el uso de ultrasonidos, condujo sólo a dos metabolitos (fenchona y anetol), la maceración realizada por ultrasonidos en 5 y 10 minutos, promovió la extracción de siete metabolitos (α -pineno, L-fenchona, estragol, anetol, β -cariofileno, α -cadinol y geranilgeraniol), mientras que el uso de ultrasonidos en tiempos superiores a 10 minutos da lugar a extractos con 4 metabolitos (α -pineno, L-fenchona, estragol). Los extractos acuosos sometidos o no al ultrasonido dieran lugar a la extracción del mismo y de cinco metabolitos (D-limoneno, L-fenchona, estragol y anetol).

Palabras clave: Cromatografía de gases; Infusiones; Tés; Hinojo; *Foeniculum Vulgare Mil.*

1. Introduction

The therapeutic actions of medicinal plants come from its bioactive metabolites-the phytochemicals. Among these substances, the mono, di and sesquiterpenes are very representative, mainly in aromatic herbs that are used by people around the world as “teas” to treat digestive diseases, anxiety and pains (Cox-Georgian et al., 2019).

Fennel (*Foeniculum vulgare Mill*) is a species originated from the southern Mediterranean region and is also found in northern, eastern, and western hemispheres, specifically in Asia, North America, and Europe (Al-Snafi, 2018). The major metabolites of *Foeniculum vulgare* are the phenolic compounds, and the monoterpenes trans-anethole, estragole and fenchone. (Kishore e Verma, 2022). The antioedematogenic and antihypernociceptive effects of anethole have been proven, being the peripheral antinociceptive action, promoted by the decrease in the synthesis or release of inflammatory mediators (Silveira and Sá et al., 2017). The gastroprotective activity of fenchone has been proven, related to cytoprotective, antioxidant and immunoregulatory mechanisms (Guedes, 2018). The anti-inflammatory, antioxidant, venous relaxer and gastroprotective activities of the estragole, are reported, the latter being related to antisecretory, cytoprotective, antioxidant and immunoregulatory mechanisms (Alves Junior, 2019; Farid et al., 2020).

Due to their low polarity, monoterpenes are extracted more efficiently when organic solvents or when heating methods are employed. Both of then heating process and the use of organic solvents are against the “green chemical” principles. To be in accordance with these principles, the extraction should be performed promoting the substitution of organic solvents by solvents of lower toxicity and the use of chemical methods that promote the lowest possible energy consumption (Grazhdannikov, 2018; Penido, 2022; Lenardão et al., 2003; Chemat et al., 2012).

The evaluation of the use of ultrasound as a viable strategy for obtaining extracts rich in terpenes becomes interesting, since this resource can promote increased rates of mass transfer by diffusion between membranes. The ultrasound waves favor the penetration of the solvent into the product by the disruption of cell walls produced by acoustic cavitation (Singla e Sit, 2021), which is produced by the formation and collapse of bubbles. The implosion of cavitation bubbles generates high turbulence, high velocity, particle collision, and has high pressure and temperature at the point of bubble collapse (Hiremath et al., 2020). The ultrasonic system allows the use of milder operating conditions and offers several advantages in terms of yield, productivity, selectivity and reduced use of chemical solvents, making it one of the main "eco-friendly processes". Ultrasonic

extraction has been used to extract components with potential biological activities and isolation of volatile components from natural products at room temperature with organic solvents (Tian et al., 2021).

The present study intends to verify if the use of ultrasound can cause an improvement to extractive processes of the *Foeniculum vulgare* Mill by maceration in ethyl acetate and by infusion in water.

2. Methodology

2.1 Purchase of plant material

Fennel leaves were collected in the city of Contagem - MG- Brazil, under the GPS geographic coordinates (-19.987501, -43.998716), at 7:00 am on February 10, 2019.

2.2 Treatment of plant material

Two hours after collection, the plant material was manually fragmented and homogenized.

2.3 Extraction by maceration in ethyl acetate

For preparation, of each extract, the mass of 1.0 g of the plant material was measured and transferred to an amber flask, in which 5.0 mL of ethyl acetate was added. Four duplicates of this systems were left to stand at room temperature by periods of 5, 10, 20, and 30 minutes. Other four duplicates were submitted to ultrasonification by periods of 5, 10, 20, and 30 minutes. After the maceration period, the systems were filtered using a pasteur pipette with a cotton swab.

For sonication of the extracts, a Schuster L-220 ultrasonic washer with 1.0 L capacity and 20 kHz frequency was used.

2.4 Extraction by water infusion

For preparation, of each extract, a mass of 1.0 g of the plant material was measured and transferred to an amber flask, in which 5.0 mL of boiling water was added. Two duplicates of these systems were left to stand at room temperature for periods of 5 and 20 minutes and other two duplicates were submitted to ultrasound by 5 and 20 minutes, respectively. After the infusion period, the systems were filtered using a pasteur pipette with a cotton swab.

2.5 Dispersive Liquid-Liquid Microextraction

To each eppendorf was added: 750 μ L, of the aqueous extract prepared by water infusion and 1 mg of sodium chloride PA, 250 μ L of dichloromethane PA and 100 μ L of ethanol PA. The mixture was vortexed for 1 minute and then centrifuged for 2 minutes. With the aid of a syringe, the organic phase was collected and transferred to a microvial to be submitted to gas chromatography coupled to mass spectrometry (GC-MS) analysis. (Martins et al., 2012).

2.6 Chromatographic Analysis

Terpenes are frequently detected and identified by gas chromatography coupled to mass spectrometry (GC-MS), being one of the most indicated and used for this purpose due to its efficiency, ease and speed of execution in addition to providing representative results (Niculau; Lima and Silva, 2021; Garcia et al., 2021).

To perform the chromatographic analysis of the samples it was used an apparatus (Agilent Technology/model 7890A, coupled to the selective mass detector Agilent Technology/model 5975C inert MSD triple-axis Detector) with a fused silica capillary column DB-5MS (30m x 0, 25mm x 0.25 μ m), maintaining helium flow, as a carrier gas, heating with a programmed

temperature of 60°C for 2 minutes up to 110°C at 6°C min⁻¹, finally up to 180°C at 10°C min⁻¹, maintained for 5 minutes.

The identification of the substance related to each peak obtained in the chromatograms was performed according to the Kovats method (Adams, 2017) and by comparing the mass spectra obtained for each substance with the mass spectra available in the device library, considering a 95% match.

3. Results and Discussion

Considering all the extracts, eight monoterpenes were identified: α -pinene, L-fenchone, estragole, anethole, β -caryophyllene, α -cadinol, geranylgeraniol and limonene. The retention time and the Kovats index obtained to each metabolite are present at Table 1. Metabolite that are present in each extract are specified in Table 2.

Table 1. Retention time and Kovats index for the metabolites identified in the fennel samples.

Metabolite	Solvents			
	Ethyl Acetate		Water	
	KT	KC	KT	KC
α -pinene	930	914	-	-
D- limonene	-	-	1020	1015
L-fenchone	1083	1083	1083	1083
estragole	1177	1193	1177	1193
anethole	1264	1271	1264	1271
β -caryophyllene	1424	1421	-	-
α -cadinol	1641	1614	-	-
geranylgeraniol	2201	**	-	-

KT = Theoretical Kovats; KC = Kovats calculated; - absent; ** not identified by Kovats. Source: Authors.

The Kovats index that were experimentally obtained to the metabolites present in extracts were in accordance with the theoretical Kovats index.

Extractions by maceration in ethyl acetate of fresh fennel leaves without ultrasound led to the extraction only of L-fenchone and anethole, even with variation of the extraction time. Although, the extractions performed by maceration, by a period of 5 minutes, with the aid of ultrasound, led to the extraction of α -pinene, L-fenchone, estragole, anethole, β -caryophyllene, α -cadinol, and geranylgeraniol, highlighting the fact that ultrasonification favors the extraction of terpenes in ethyl acetate. But when the ultrasound exposition takes time periods upper than 5 minutes, β -caryophyllene, α -cadinol, and geranylgeraniol were not observed. Babicz (2009) and Chemat and collaborators (2011) said that ultrasound can improve the approximation between enzymes and substrates as well as it can cause changes at the conformation of enzymes active sites that leads to the breaking of the most labile chemical links of the molecules. In this way, the use of ultrasound in extract preparation can have ambiguous effects: by one side it can improve the mass transfer, but in other way, it can cause the degradation of the metabolites, according to the exposure time period.

Table 2. Metabolites identified in the extracts prepared by maceration in ethyl acetate and by water infusion.

	Maceration in ethyl acetate								Water infusion			
	Without ultrasonification				With ultrasonification				Without ultrasonification		With ultrasonification	
Contact time (min.)	5	10	20	30	5	10	20	30	5	20	5	20
α -pinene	-	-	-	-	+	+	+	+	-	-	-	-
L-fenchone	+	+	+	+	+	+	+	+	+	+	+	+
estragole	-	-	-	-	+	+	+	+	+	+	+	+
anethole	+	+	+	+	+	+	+	+	+	+	+	+
β -caryophyllene	-	-	-	-	+	-	-	-	-	-	-	-
α -cadinol	-	-	-	-	+	-	-	-	-	-	-	-
geranylgeraniol	-	-	-	-	+	-	-	-	-	-	-	-
D-limonene	-	-	-	-	-	-	-	-	+	+	+	-

(+) presence; (-) absence of the metabolite in the chromatogram obtained from the ethyl acetate extracts. Source: Authors.

In the fresh leaf extracts infused in water, without ultrasound by time periods of 5 and 20 minutes and the extract obtained by ultrasound by 5 minutes, the bioactive metabolites D-limonene, L-fenchone, estragole and anethole were identified, while in the extract obtained by infusion by 20 minutes, by using ultrasound, limonene was not present. These results showed that the ultrasound don't favor the extraction in water and 20-minute extraction time with ultrasound favored the degradation of D-limonene. The behavior of monoterpenes demonstrated that these compounds have differentiated stabilities with specific degradation times when exposed to ultrasound waves. Our results corroborate the data presented by Xu and Pan (2013) and Anese et al. (2013), which demonstrated that lycopene by ultrasound extraction in different time periods, remaining stable up to 10 minutes of extraction. Geranylgeraniol has a similar structure to lycopene and, therefore, showed the same behavior when ultrasound was used in its extraction, but its stability was guaranteed only with 5 minutes of extraction. It is important to consider that the choice of the extraction time should be rational, because the longer the exposure time of the compounds to ultrasound, the greater the possibility of degradation of the compounds.

Concerning the pharmacological actions of the metabolites present in aqueous extracts, it can be observed the presence of the anethole and D-limonene, for which the antinoceptive action is proven, as discussed by Silveira and Sá et al. (2017), the presence of l-fenchone that has antinoceptive action and the presence of estragole that has gastroprotective action, as reported by Guedes (2018) and Alves Junior (2019).

4. Conclusion

Maceration of fresh leaves of *Foeniculum vulgare* Mill in ethyl acetate for five minutes without the use of ultrasound promoted the extraction of two metabolites while the use of ultrasound promoted the extraction of seven metabolites but in the extracts prepared by longer maceration time, only three metabolites remained. So, it is possible to conclude that even the use of ultrasound can improve the sesquiterpenes extraction in ethyl acetate, it also can promote the degradation of some if the time is longer than 5 minutes.

The aqueous infusion of fresh leaves, with and without ultrasonication, promoted the extraction of the same five metabolites: d-limonene, l-fenchone, estragole, and anethole, while the process takes long 5 minutes. Upper time them 5 minutes of ultrasonification, promoted the degradation of the limonene, lead to the conclusion that ultrasonification doesn't causes any improvement in water's extract preparation process.

The use of ultrasound was able to improve the extraction by maceration of the *Foeniculum vulgare* Mill in etil acetate when it was performed by 5 minutes.

From the results we hope to research for new green methodology articles that are efficient for the extraction of terpenes from medicinal plants.

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