Tropical Journal of Pharmaceutical Research January 2019; 18 (1): 55-60
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v18i1.9

Original Research Article

Identification of heat-resistant chemical components of *Ferula elaeochytris* root extracts by gas chromatography-mass spectrometry

Nadire Eser[1], Atila Yoldas[2]
[1]Department of Pharmacology, [2]Department of Anatomy, Faculty of Medicine, Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey

For correspondence: **Email: esernadire01@hotmail.com*

Sent for review: 25 July 2018 *Revised accepted: 19 December 2018*

Abstract

Purpose: *To determine the chemical composition of Ferula elaeochytris, a plant with traditional medicinal uses, and to investigate its potential benefits.*
Methods: *Ferula elaeochytris specimens were collected from Engizek plateau of Kahramanmaras, Turkey in early June 2017, and dried in a cool and dry place at room temperature. Once dried, they were ground into a powder using a plant grinder. A Soxhlet extractor was employed for extraction of the powdered roots using diethyl ether solvent for 4 h, and then subsequently evaporated using a rotary evaporator. The extract was analysed by gas chromatography–mass spectrometry (GC-MS) to reveal the components of F. elaeochytris.*
Results: *Khusinol, ferutinin and beta-ionone, components with anti-inflammatory phytoestrogen, antiproliferative and antioxidant activities, were found in high levels (25.9, 13.9 and 22.9 %, respectively) in F. elaeochytris root extract. In addition, α-pinene, which has anti-inflammatory, bronchodilator, hypoglycaemic and anticarcinogenic effects, was found at lower levels (12.9 % or less).*
Conclusion: *The results reveal the presence of some therapeutically beneficial components of F. elaeochytris root extract, including compounds with antioxidant and anti-inflammatory effects.*

Keywords: *Ferula elaeochytris, Khusinol, Ferutinin, Beta-ionone, Anti-inflammatory Phytoestrogen, Antiproliferative, Antioxidant*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

The significance of phytotherapy is recognised worldwide. In some industrilised regions, most of the population utilise at least one complemetary and alternative medicine [1]. *Ferula L*. is a perennial genus of the Apiaceae family, represented by almost 185 species. Twenty-three of these species are part of the flora of Turkey, 12 of which are endemic [2]. Species of this genus are known as Çağşır, Çanşur, Hiltik, Helizan, or Kasnı in different regions of Anatolia. These species have been traditionally used as an aphrodisiac in the southeastern regions of Turkey, as well as in some other countries [2,3]. Moreover, the leaves and roots of this plant have been used for centuries to encourage the mating of goats and sheep [2].

Studies on some species of *Ferula* contain tannins, saponins, terpenes, amylum, essential oils, and alkoloids and have suggested that, in addition to their aphrodisiac effect [4], these species also contain components, namely α-pinene and germacrene B, which have potent antioxidant and anti-inflammatory effects. They have also been reported to display cytotoxic, antidiabetic, antimicrobial, antifungal, antiulcerative and hepatoprotective effects in humans, as well as a relaxant effect on the corpus cavernosum [5-8].

Ferula elaeochytris Korovin is a species of *Ferula* that is found throughout Syria, Lebanon and the eastern Mediterinean provinces of Turkey, such as Hatay and Kahramanmaras [9]. In a previous study, root extract of *Ferula elaeochytris* collected from the Engizek plateau in Kahramanmaras was found to have positive effects on diabetes and infertility [10]. Although commercial products derived from *Ferula elaeochytris Korovin* are abundantly consumed, there is limited information about the composition of the plant. Thus, the aim of this study was to evaluate the chemical composition of *Ferula elaeochytris Korovin* from Kahramanmaras, Turkey.

EXPERIMENTAL

Plant material

Roots of *F. elaeochytris* were collected from the Engizek plateau (Kahramanmaras) in June 2017. The specimens were authenticated by Prof Dr Ilhan Uremis (a taxonomist). A voucher specimen has been kept in the herbarium of the Department of Plant Protection, Faculty of Agriculture, Hatay Mustafa Kemal University, Turkey.

Preparation of *Ferula elaeochytris* roots

F. elaeochytris roots investigated in this study were collected from the Engizek plateau, Kahramanmaras, in the first week of June 2017. Soil and foreign materials on the outer surface of the roots were removed, and roots were dried on a clean, dry surface to ensure no decomposition occurred. Dried specimens were then pulverised through a 1-mm sieve. Ground samples were placed in thimbles and covered with cotton.

Ferula elaeochytris extraction

A solvent extraction method was used for the extraction of *F. elaeochytris* roots. Extraction was performed using a Soxhlet extractor, with each apparatus volume being 250 mL [11]. The thimbles containing 20 g of ground *F. elaeochytris* root were placed into the extraction tube of the Soxhlet extractor. A boiling flask with a known weight (M1) was placed under the extraction tube. Approximately 320 mL of diethyl ether (Merch, Germany) was added to the extraction tube (enough solvent to be emptied via the siphon and filled up halfway) and extracted in the re-cooling system at 60 ºC for 4 h (Table 1).

At the end of the extraction, the flasks containing the solvent were connected to an evaporator (Heidolph WB 2000) to remove the solvent. The samples, removed from the evaporator were kept in the incubator at 60 ° C for 30 minutes to completely blow the solvent in the event of possible residual solvent, which was then cooled down to room temperature in a desiccator and weighed (M_2). Yield (Y) was computed as in Eq 1.

$$Y\ (\%) = \{(M_2 - M_1)/m\}100 \quad \ldots\ldots\ldots \quad (1)$$

where M_1 is the weight of the flask (g), M_2 the weight of the crucible with the extract (g) and m is the weight of the dry sample (g).

Gas chromatography–mass spectrometer (GC-MS) analysis

Gas chromatography–mass spectrometry (GC-MS Clarus 500; Perkin Elmer Inc., Waltham, MA, USA) was performed for analysis. Sample preparation for injection to the instrument was done as 0.1 mL of extract with vortexed 1 mL of n-hexane. For GC-MS injection, 1 μL of sample was collected in an injector and directly injected to the GC/MS instrument. The results are expressed as a percentage. The analysis conditions for GC-MS are given in Table 1.

Statistical analysis

The relative amount of the separated compounds, expressed as a percentage, were calculated from the total ion chromatograms using the computerised integrator. Only components with a relative percentage over 0.1 % (on a polar column) are reported here. The results were calculated as a percentage using Microsoft Excel version 2013. The data are reported as the mean of triplicate measurements.

RESULTS

In our study, we analysed the root extract of *F. elaeochytris* specimens collected from the Engizek plataeu of Kahramanmaras by GC-MS.

Table 1: Gas chromatography–mass spectrometry (GC-MS) operating conditions

Variable	Operating condition
Column	SGE BPX-5 GC-column (60m×0.25 mm internal diameter, 0.25 µm) (SGE Inc., USA)
Column temperature	10 min at 60 ºC, increased to 220ºC at a rate of 4ºC per min, then kept at 220 ºC for 10 min. Increased to 250 ºC at 4 ºC per min, then kept at 250 ºC for 10 min.
Injection port temperature	240 ºC
Oven temperature	220 ºC
Carrier gas	Helium (1.5 mL/min)
Split ratio	0
Electron ionisation energy	70 eV
Mass range	35–425 m/z
Library database	Nist and Wiley
Detector	PMT (Photomultiplier)

From our findings, *F. elaeochytris* contains 39 different components (Figure 1; Table 2). Some of the components have bioactivity, considered to have anti-inflammatory (khusinol, 25.9 %), phytoestrogen (ferutinin, 13.9 %), antiproliferative and antioxidant (beta-ionone, 22.9 %), anti-inflammatory, hypoglycaemic, antioxidant and anticarcinogenic (alpha-pinene 12.9 %), antioxidant (beta-farnesene 1.3 %), anti-inflammatory, antiallergic and antiasthmatic (Drimenol 0.96 %) effects (Table 3). On the other hand, Mass spectrum of some major compounds identified in the extract (Figure 2).

DISCUSSION

Many studies have been carried out on different species of the *Ferula* genus. These studies have shown that different components may be present, and even the same *Ferula* species can contain different components when grown in different regions. These differences may be due to numerous factors, including the different environmental conditions of the region, such as the vegetation, climatic conditions, and minerals in the soil [12]. As the components of the same plant species grown in different regions may vary, and considering that these components have different effects and mechanisms of action, this may result in variations between plants with regard to their medicinal properties, such as their antioxidant, anti-inflammatory, cytotoxic and antiproliferative effects.

Although *F. elaeochytris* is widely used among Anatolian people, we could only find a single study in the literature that investigated its composition, which was focused on *F. elaeochytris* from Yayladagı region of Antakya [9]. The plants used in our study were collected from the Engizek plataeu of Kahramanmaraş, which has different enviromental conditions. We identified 39 different components of *F. elaeochytris* by GC-MS (Table 2). The study by Baser et al. identified 43 different components of *F. elaeochytris* collected from Yayladağ, Antakya, by GC-MS analysis [9].

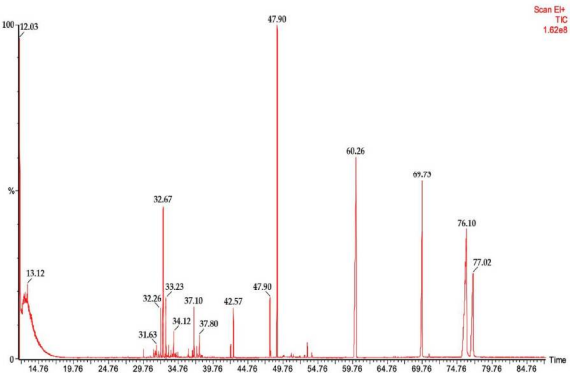

Figure 1: Chromatogram of *Ferula elaeochytris* extract

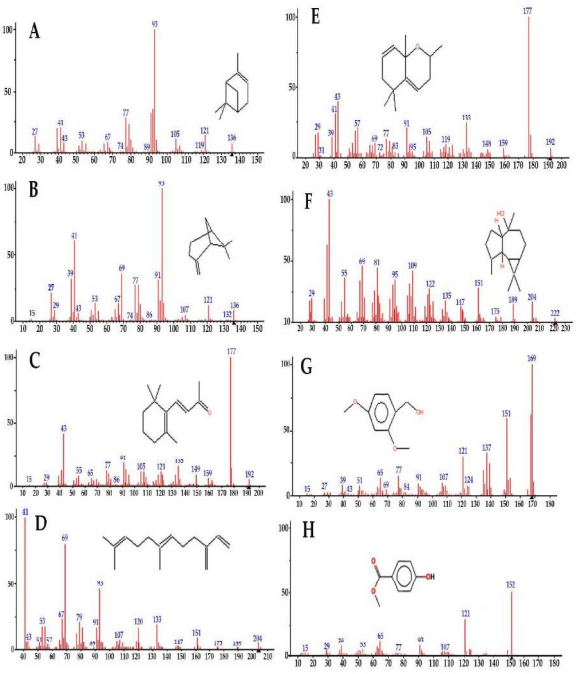

Figure 2: Mass spectrum of major compounds identified in the extract. ***Note:*** **A:** Alfa- pinene; **B:** Beta- pinene; **C:** Beta-ionone; **D:** β-Farnesene; **E:** Trans-edulan; **F:** Globulol; **G:** 2,4-Dimethoxybenzyl alcohol; **H:** Methyl paraben

Table 2: Composition of *Ferula elaeochytris* extract

RT	Area (%)	Compound	CAS no.
29.80	0.225992	γ-cadinene	39029-41-9
31.26	0.275786	9-Aristolen-1-ol	27862-07-3
31.50	0.183513	Allo-aromadendrene	25246-27-9
31.56	0.183967	(-)-|A-Gurjunene	489-40-7
31.63	0.50014	(-)-Isoledene	95910-36-4
31.95	0.168248	α-amorphone	483-75-0
32.04	0.057981	Seychellene	20085-93-2
32.26	1.355226	Beta-farnesene	18794-84-8
32.27	0.088014	Sorelline	73004-62-3
32.67	12.8970	Alfa- pinene	7785-26-4
33.23	2.65110	Beta- pinene	127-91-3
33.39	0.309169	(-)-alpha-curcumene	4176-17-4
33.72	0.238055	Homomyretenol	128-50-7
33.89	0.099362	Eudesma-3,11-diene	473-13-2
34.12	1.038253	Alpha-cendrene	469-61-4
34.33	0.108814	Propene, 2-methyl-1-(trimethylcyclopropylidene)-	14803-30-6
34.46	0,133692	(+)-Cuparene	16982-00-6
34.51	0.067404	Cadina-3,9-diene	523-47-7
34.72	0.12934	L-Caryophyllene	87-44-5
36.21	0.315649	beta-Caryophyllene oxide	1139-30-6
36.77	0.191072	(+)-Spathulenol	6750-60-3
36.87	0.196105	Beta-costal	3650-40-6
37.10	1.675901	Globulol	489-41-8
37.42	0.457788	Viridiflorol	552-02-3
37.68	0.066949	Cedrelanol	5937-11-1
37.80	0.963029	Drimenol	584-79-2
38.06	0.069892	Selinenol	1209-71-8
42.28	0.372852	3-hydroxy-.beta.-ionone	15401-34-0
42.57	1.394499	Neoclovene oxide	
47.90	1.583501	ar-Turmerone	532-65-0
48.95	13.58864	Trans-edulan	41678-29-9
50.96	0.123127	hexyl cinnamylaldehyde	101-86-0
53.27	0.663831	Phenethyl tiglate	55719-85-2
53.91	0.225136	3-Methyl-2-Butenoic Acid,2,6-Dimethylnon-1-En-3-Yn-5-Yl ester	
60.26	22.89477	Beta-ionone	79-77-6
69.73	13.947	Ferutinin (Methylparaben)	41743-44-6
70.72	0.1837	Isobutyric Acid Piperonyl Ester	5461-08-5
76.10	25.93169	Khusinol	24268-34-6
77.02	11.52541	2,4-Dimethoxybenzyl alcohol	7314-44-5

RT: Retention time; CAS no: Chemical Abstracts Service number

Table 3: Major components identified bioactivities of *Ferula elaeochytris* root extract

Compound	CAS no.	Component and activity	Reference
Khusinol	24268-34-6	Terpenoid, Anti-inflammatory	[9], [13]
Beta-ionone	79-77-6	Terpenoid Antiproliferative, antioxidant, chemopreventive	[14], [15]
Ferutinin	41743-44-6	Terpenoid, Phytoestrogen, Treatment osteoporosis, anticarcinogenic	[19-23]
Alfa- pinene	7785-26-4	Anti-inflammatory, bronchodilator, hypoglycaemic, sedative, antioxidant, anticarcinogenic	[9], [16]
Beta-farnesene	18794-84-8	Antioxidant	[17]
Drimenol	584-79-2	Anti-inflammatory, antiallergic and antiasthmatic	[25]

Beta-farnesene (1.35 %) and alpha-curcumene (0.309 %) were found at higher levels in the root extracts of plants collected from Kahramanmaraş compared to plants from Antakya [9], while caryophyllene oxide (0.315 %) was present at lower levels than plants from Antakya. Furthermore, beta- (2.651 %) and alpha-pinene (12.8 %) levels were similar between samples from the two regions.

Previous evaluations have confirmed the presence of substances in *F. elaeochytris* with significant medicinal effects. The most important compound identified is khusinol, a member of the terpenoid class, which has been identified in many species of the plant, and its chemical structure revealed [13]. Although we found a high khusinol (25.93 %) content in *F. elaeochytris* from Kahramanmaraş, there was no mention of this compound in the study of *F. elaeochytris* from Yayladag, Antakya [9].

One of the most interesting findings of the present study was the identification of beta-ionone, a terpenoid, in the samples. Beta-ionone has been approved as a safe food additive by the US Food and Drug Administration in 2015. Moreover, beta-ionone has chemoprevention effect of rat mammary carcinogenesis [14]. It also plays an important chemopreventive role in lung cancer [15]. While beta-ionone was detected at high levels (22.89 %) in our study, Başer *et al*. [9] did not report the presence of beta-ionone in their study on the composition of *F. elaeochytris* from Antakya. Several investigators have reported the presence of alpha-pinene in *Ferula* species. This compound has been reported to have a variety of interesting pharmacological properties such as strong anti-inflammatory, bronchodilator, hypoglycaemic, sedative, antioxidant and broad-spectrum antibiotic activities [16]. Alpha-pinene was detected in the investigated samples, consistent with previous studies [9].

The leaves and roots of *F. elaeochytris* have been used to encourage the mating of goats and sheep, and people have traditionally consumed the plant as an herbal infusion as an aphrodisiac. In addition, in a study on erectile dysfunction in diabetic rats, *F. elaeochytris* root extract collected from the Engizek plateau of Kahramanmaraş was found to have positive effects on both diabetes and infertility [10]. In our study, beta-farnesene was detected in *F. elaeochytris* root extract. A previous study by Fırat *et al*. [17], reported that plants containing this substance have antioxidant effects and a study by Caurnel *et al* [18] showed that they stimulate sex drive.

Ferutinin, a terpenoid, has been previously detected in *Ferula* spp. such as *F. hermonis* [19], *F. communis* [20] *and F. elaeochytris* [21]. The oestrogen-like effects of the phytoestrogen ferutinin has been demonstrated in experimental studies, particularly in relation to its apoptotic properties, as well as its influence on calcium mobilisation, mitochondrial permeability and beneficial effect on osteoporosis [22, 23]. Ferutinin was identified in our specimens, similar to previous studies on *Ferula* spp. [19-21]. Unlike the findings of our study, Başer *et al*. [9] did not identify ferutinin in *F. elaeochytris* collected from Hatay.

One of the most important substances identified in the root extract of *F. elaeochytris* in the current study was curcuminine. This compound has been shown to have a wide spectrum of biological activities, including anti-inflammatory, antimutagenic, fertility, anticoagulant, anticarcinogenic, antiulcer, antibacterial, antidiabetic, antifungal, antioxidant, antiprotozoal, antiviral, anti-Alzheimer antivenom, and hypocholesteremic activities [24]. Furthermore, based on interviews with the locals, this plant has also been traditionally used for headaches and the common cold. Drimenol, a substance found in plants which is known to have anti-inflammatory, antiallergic and antiasthmatic effects [25], was present in low concentrations in our materials.

CONCLUSION

The findings of the current study indicate the presence of several potentially beneficial components in *F. elaeochytris* root extract (with antioxidant, anti-inflammatory, antidiabetic, anticarcinogenic and antiproliferative effects). These findings can form the basis of future preclinical and clinical trials to evaluate the potential application of *F. elaeochytris* in clinical practice.

DECLARATIONS

Acknowledgement

The authors would like to thank Dr Yılmaz Ucar, Department of Seafood Processing Technology, Faculty of Fisheries, University of Cukurova, Adana for assistance with GC-MS analysis.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

REFERENCES

1. *Sarisen O, Caliskan D. Fitotherapy: Herbal Medicine, Attetion of in Primary Health Care. Sted 2005; 14 (8): 187.*
2. *Baytop T. Therapy with medicinal plants in Turkey-Pastand Present. Nobel Medical Publication. 2nd ed. Istanbul; 1999. p. 241-244.*
3. *Sagıroglu M, Duman H. Rediscovery of Ferula anatolica and Ferula drudeana (Apiaceae) from Turkey. Biol Diver Conserv 2011; 4: 191-197.*
4. *Homady MZ, Khleifat KM, Tarawneh KK, Al-Raheil IA. Reproductive toxicity and fertility effect of Ferula hermonis extracts in mice. Theriogenology 2002; 57: 2247-2256.*
5. *Kavoosi G, Tafsiry A, Ebdam AA, Rowshan V. Evaluation of antioxidant and antimicrobial activities of essential oils from Carum copticum seed and Ferula assafoetida latex. J Food Sci 2013; 78: 356-361.*
6. *Kassis E, Fulder S, Khalil K, Hadieh B, Nahhas F, Saad B, Said O. Efficacy and Safety Assessments of Ferula assa-foetida L., Traditionally used in Greco-Arab Herbal Medicine for Enhancing Male Fertility, Libido, and Erectile Function. Open Complement. Med. J 2009; 1: 102-109.*
7. *Kareparamban JA, Nikam PH, Jadhav AP, Kadam VJ. Ferula foetida Hing A Review. Res J Pharm Biol Chem Sci 2012; 3: 775.*
8. *Ozturk B, Gur S, Coskun M, Kosan M, Erdurak CS, Hafez G, Gonulalan U, Cetinkaya M. A new relaxant on human corpus cavernosum: Ferula gosyriaca root extract. Afr J Pharm Pharmacol 2012; 6: 2652-2656.*
9. *Başer KHC, Özek T, Demirci B, Kurkcuoglu M, Aytac Z, Duman H. Composition of the essential oils of Zosima absinthi folia (Vent.) Link and Ferula elaeochytris Korovin from Turkey. Flav Fragr J 2000; 15 (6): 371-372.*
10. *Eser N. The effect of ferula elaeochytris (çakşir) root extract on erectile dysfunction in diabetes mellitus induced rats. Thesis, Adana, Turkey 2016; https://tez.yok.gov.tr/UlusalTezMerkezi/tezSorguSonucYeni.jsp*
11. *Bimakr M, Rahman RA, Taip FS, Ganjloo A, Salleh LM, Selamat J, Hamid A, Zaidul ISM. Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (Menthaspicata L.) leaves. Food Bioprod. Process 2011; 89: 67-72.*
12. *Dong JE, Ma XH, Wei Q, Peng SB, Zhang SC. Effects of growing location on the contents of secondary metabolites in the leaves of four selected superior clones of Eucommia ulmoides. Ind Crop Prod 2011; 34: 1607-1614.*
13. *Belhassen E, Filippi JJ, Brevard H, Joulain D, Baldovini N. Volatile constituents of vetiver: a review. Flavour Fragr J 2015; 30: 26-82.*
14. *Yu SG, Anderson PJ, Elson CE. Efficacy of beta-ionone in the chemoprevention of rat mammary carcinogenesis. J Agric Food Chem 1995; 43: 2144-2147.*
15. *Asokkumar S, Naveenkumar C, Raghunandhakumar S, Kamaraj S, Anandakumar P, Jagan S, Devaki T. Antiproliferative and antioxidant potential of beta-ionone against benzo(a)pyrene-induced lung carcinogenesis in Swiss albino mice. Mol Cell Biochem 2012; 363: 335–345.*
16. *Mercier B, Prost J, Prost M. The essential oil of turpentine and its major volatile fraction (alpha- and beta-pinenes): a review. Int J Occup Med Environ Health 2009; 22: 331–342.*
17. *Fırat Z, Demirci F, Demirci B. Antioxidant Activity of Chamomile Essential Oil and Main Components. Nat. Volatiles & Essent. Oils 2018; 5(1): 11-16.*
18. *Morgan C, Thomas RE, Ma W, Novotny MV, Cone RD. Melanocortin-5 Receptor Deficiency Reduces a Pheromonal Signal for Aggression in male mice. Chem. Senses 2004; 2: 111–115.*
19. *Zanoli P, Rivasi M, Zavatti M, Brusiani F. Activity of single component of Ferula hermonis on male rat sexual behaviour. Int J Impot Res 2005; 17: 513–518.*
20. *Sacchetti, G, Appendino G, Ballero M, Loy C, Poli F. Vittae fluorescence as a tool to differentiate between poisonous and non-poisonous populations of giant fennel (Ferula communis) of the island of Sardinia (Italy). Biochemical Systematics and Ecology. 2003; 31: 527-534.*
21. *Duru M, Sahin A. Effects of dietary male and female Ferula eleaochytris Powder on Growth Performance and Body Components of Broiler Chicks. Turkish Journal of Agriculture – Food Science And Technology, 2015; 3: 413-417.*
22. *Ferretti M, Bertoni L, Cavani F. Influence of ferutinin on bone metabolism in ovariectomized rats. II: Role in recovering osteoporosis. J. Anat 2010; 217: 48–56.*
23. *Appendino G, Spagliardi P, Cravotto G, Pocock V, Milligan S. Daucane phytoestrogens: a structure-activity study. J Nat Prod 2002; 65: 1612–1615.*
24. *Shehzad A, Lee Y. Curcumin: Multiple molecular targets mediate multiple pharmacological actions: A review. Drugs Fut. 2010; 35: 113-120.*
25. *Lago JHG, Carvalho LAC, Silva FS, Toyama DO, Romoff F, Romoff P. Chemical composition and anti-inflammatory evaluation of essential oils from leaves and stem barks from Drimys brasiliensis Miers (Winteraceae). J Braz. Chem. Soc. 2010; 9: 1760-1765.*