UNIVERSIDADE DE SÃO PAULO

FACULDADE DE CIÊNCIAS FARMACÊUTICAS DE RIBEIRÃO PRETO

Metabolomics studies of wild edible plants from Asteraceae family

Estudos metabolômicos de plantas alimentícias selvagens da família Asteraceae

JOLINDO ALENCAR FREITAS

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Doctoral thesis presented to the Graduate Program of Pharmaceutical Sciences of School of Pharmaceutical Sciences of Ribeirão Preto/USP for the degree of Doctor in Sciences.

Concentration Area: Natural and synthetics products

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Supervisor**:** Prof**.** Dr. Fernando Batista da Costa

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ABSTRACT

FREITAS, J. A. **Metabolomics studies of wild edible plants from Asteraceae family. 2020. 72 p. Thesis (Doctoral).** Faculdade de Ciências Farmacêuticas de Ribeirão Preto – Universidade de São Paulo, Ribeirão Preto, 2020.

The plant family Asteraceae has important edible species, such as lettuce and chicory, which are widely cultivated around the world. Studies have demonstrated the importance to ingest wild and/or spontaneous plant species as salads or condiments, since they are sources of a wide variety of nutrients and secondary metabolites. Unfortunately, edible wild species ethnobotanical studies presented the use of species with chronic toxic compounds as pyrrolizidine alkaloids or unclear phytochemistry as *E. fosbergii*. This study aims to demonstrate, through LC-MS untargeted metabolomics analysis of plants, possible risks and advantages of including 11 Asteraceae wild edible leaves in population diet. Initially, *E. fosbergii* had its chemical profile studied, which showed for the first time in this species the occurrence of 28 compounds, including pyrrolizidine alkaloids, flavonoids and cinnamic acid derivatives. At second, *E. fosbergii* and *E. sonchifolia* had their metabolic profiling compared, revealing that *E. fosbergii* accumulates more pyrrolizidine alkaloids than *E. sonchifolia*, with main importance to emiline compound, present mainly in the first species. Then, the influence of soil composition and phenology were studied in both species, revealing the situation in which each plant accumulates certain classes of compounds. Apart from that, other nine species, including wild and domesticated crop species, had their LC-MS chemical profile compared. It was possible to observe that *Bidens pilosa*, *Galinsoga parviflora*, *Acmella oleraceae* samples had different chemical profiles from Cichorieae tribe samples. *Cichorium intybus* and *Youngia japonica* species had unique chemical profiles and were distinguished from other four species. *Lactuca canadensis*, *Sonchus oleraceus* and *Cichorium endivia* demonstrated a similar chemical profile among each other and with a few *L. sativa* samples. The main discriminant compounds were linked to the chemotaxonomic information of each tribe. In conclusion, these findings have not only demonstrated the importance of chemotaxonomy in Asteraceae but also presented that the studied wild edible species could bring a variety of secondary metabolites to population. At last, this study confirmed hypothesis of pyrrolizidine alkaloids presence in *E. fosbergii*, which is a risk to the population due to the toxicity of this class of secondary metabolites.

Keywords: Asteraceae, metabolomics, Wild edible plants, Unconventional edible plants, pyrrolizidine alkaloids

RESUMO

FREITAS, J. A. **Estudos metabolômicos de plantas alimentícias selvagens da família Asteraceae. 2020. 72 p. Tese (Doutorado).** Faculdade de Ciências Farmacêuticas de Ribeirão Preto – Universidade de São Paulo, Ribeirão Preto, 2020.

A família Asteraceae possui importantes plantas alimentícias, como alfaces e chicórias, as quais são extensamente cultivadas pelo mundo todo. Estudos têm demonstrado a importância de se incluir espécies de plantas selvagens e/ou espontâneas na alimentação, nas formas de salada ou condimento, a fim de incrementar a ingestão de determinados nutrientes e metabólitos secundários. Infelizmente, alguns estudos de espécies alimentícias selvagens relatam o uso de plantas com presença de substâncias tóxicas, como alcaloides pirrolizidínicos, ou uso de espécies com fitoquímica pouco estudada, como *Emilia fosbergii*. Este estudo tem o objetivo de demonstrar, através de estudos metabolômicos não-direcionados por CL-EM possíveis riscos e vantagens de se incluir 11 espécies de plantas selvagens na alimentação da população. Inicialmente, *E. fosbergii* teve o perfil químico estudado e 28 substâncias foram descritas pela primeira vez nesta espécie, incluindo alcaloides pirrolizidínicos, flavonoides, e derivados do ácido cinâmico. Com isso, comparou-se o perfil metabólico de *E. fosbergii* com *E. sonchifolia,* revelando que *E. fosbergii* acumula mais alcaloides pirrolizidínicos que *E. sonchifolia*, com destaque ao alcaloide pirrolizidínico emilina, presente predominantemente na primeira espécie. Na sequência, ainda foi estudada a influência da composição do solo e do florescimento no perfil químico das duas espécies, que revelou condições em que cada espécie acumula certas classes de substâncias. Além disso, nove espécies, entre selvagens e domesticadas, tiveram o perfil químico obtido por CL-EM comparado. Foi possível observar que amostras das espécies *Bidens pilosa, Galinsoga parviflora e Acmella oleracea* tiveram perfil químico muito diferente de amostras da tribo Cichorieae. *Cichorium intybus* e *Youngia japonica* apresentam perfil químico únicos e distintos das outras quatro espécies. *Lactuca canadensis, Sonchus oleraceus* e *Cichorium endivia* demonstraram perfil químico similar entre si e com algumas amostras de *L. sativa*. Os principais discriminantes foram associados à informação quimiotaxonômica de cada tribo. Concluindo, estes resultados não apenas demonstram a importância da quimiotaxonomia em Asteraceae como também apresenta que o uso de algumas dessas espécies poderia contribuir com obtenção de uma variedade de metabólitos secundários para a população. Por último, este estudo confirmou a hipótese da presença de alcaloides pirrolizidínicos em *E. fosbergii*, o que é um alerta de risco à população devido à toxicidade dessa classe de metabólitos secundários.

Keywords: Asteraceae, metabolômica, plantas selvagens alimentícias, Plantas Alimentícias Não Convencionais (PANCs), alcaloides pirrolizidínicos.

1 INTRODUCTION

Food security and wild edible plants

The Food and Agriculture Organization (FAO) consider food security one of the biggest challenges for humanity, since it is estimated that over 820 million people worldwide were in chronic hunger situation in 2018 (FAO et al., 2019). That means that one in nine people have insufficient daily calorie intake and are uncapable to have a normal, active, and healthy life. Added to this number are 2 billion people, around a quarter of world population (FAO et al., 2019), whose daily access to food is uncertain or insufficient or who can only access food with compromised quality. In contrast to this information, the same document shows that world population suffering from obesity also reaches 2 billion people and contributes to 4 million of deaths yearly. The aforementioned numbers represent the current world situation on food security, however it could still be worsened in emergency situations, as conflicts, wars or environment disasters.

Despite seeming to be contradictory, undernourishment and obesity coexistence are very usual in poor regions and are deeply linked since both are signs of malnutrition (FAO et al., 2019; VILLENA-ESPONERA; MORENO-ROJAS; MOLINA-RECIO, 2019). Undernutrition during pregnancy or on infants could lead to stunt physical development or metabolism disorders that manifest as obesity or chronic diseases, as well as social, mental and cognition compromised levels (BLACK et al., 2004; LIU et al., 2017; VEENA et al., 2016). Malnutrition includes vitamin and minerals deficiency and is one of the biggest humanity challenges since it is generated as a consequence of health, educational, agriculture, social and economic politics (FAO et al., 2019). Obesity is one example of the connection between economy and food insecurity, since nutritive foods are expensive and processed foods (which are rich in fats, salt and sugar) are very cheap (FAO et al., 2019; VILLENA-ESPONERA; MORENO-ROJAS; MOLINA-RECIO, 2019).

Fighting against poverty is one approach, but there are other alternatives to combat malnutrition worldwide. Malnutrition could be a sign of an inadequate food system and alternatives should include diverse sectors as agriculture, transport, education and economy (FAO et al., 2019; KINUPP, 2007). Since meat-based food are expensive, and unaffordable to low-income families, an alternative to help general population to reach good nutrition parameters could be the cultivation of native plants or collect of spontaneous foods, both considered as wild food. A huge variety of grains, fruits, vegetables and even mushrooms are used worldwide as food, but just a limited number of species are used as commercial domesticated crops (BALDERMANN et al., 2016; KINUPP, 2007; KUNKEL, 1984). There is no catalogue with all edible plant species but it is theorical estimated that around 10,000 plant species would be eatable (BALDERMANN et al., 2016; KUNKEL, 1984). Even with this variety of eatable plant species, most countries economy and food production rely in just a few domesticated crops used in all parts of the world, mainly potato, rice, wheat, and soy. Adaptation of these four species in different environments worldwide generates loss of characteristics and even facilitate propagation of diseases (FAO et al., 2019; KINUPP, 2007). Native and spontaneous plants are normally used in sustainable agriculture systems and could be an interesting alternative to food production because they are 1) resistant to diseases (reducing the use of pesticides), 2) adapted to different climates, 3) source of a variety of nutrients and bioactive compounds, which makes them a viable option for trades, leading to economic growth (BALDERMANN et al., 2016).

The collection of wild plants is part of humanity development and is still kept in a number of regions and communities (SCHULP; THUILLER; VERBURG, 2014). Indigenous and traditional plants from native communities are investigated in search of a variety of nutrients, which could be used to complement dietary intake or as supplement (KINUPP, 2007; SHIN et al., 2018). A deeper knowledge on native edible plants could be a difference to survive in scarcity times, as shown in an ethnobotanical survey with elderlies Dutch (VORSTENBOSCH et al., 2017). In this study, authors rescued elderly knowledge about wild plants eaten by population in Netherlands during famine caused by World War II and state that this knowledge seems restricted to elderly population, having been erased from youngers.

Not just used in scarcity periods, the use of native and wild food is a tradition in certain locals. In Europe, hunts, fruits, mushrooms, berries and vegetables collection are part of popular alimentation and even a form of population entertainment in a few regions (SCHULP; THUILLER; VERBURG, 2014). Wild food collection, beyond fulfilling alimentation needs, becomes a recreational activity, produce entertainment, also incentives physical activity, and promotes a sustainable and integrated community. There is an estimate that in Europe around 65 million people collect wild foods and around 100 million people consume wild food (SCHULP; THUILLER; VERBURG, 2014).

These habits also were associated to Mediterranean population, which had lower risks of chronic diseases, as coronary-heart disorders, strokes (ROSATO et al., 2019; SCODITTI et al., 2012), development of cancer (SCHWINGSHACKL et al., 2017; TRICHOPOULOU; LAGIOU; KUPER, 2000), and even cognition and neurological diseases as depression and Alzheimer (PSALTOPOULOU et al., 2013; SERRA-MAJEM et al., 2019; SINGH et al., 2014). A Systematic review and meta-analysis study confirms association between Mediterranean Diet to lower levels of cancer mortality and risk of several cancer types, which includes colorectal, liver, gastric, breast, head and neck, gallbladder and biliary tract cancer (SCHWINGSHACKL et al., 2017). These effects were mainly attributable to higher intake of fruits, grains, and vegetables consumption. Another systematic review relates also evidences of Mediterranean Diet to lower risk of cardiovascular diseases, which include coronary diseases and ischemic stroke (ROSATO et al., 2019). Major cause to this healthier population characteristic would be the diet, which use big amounts of olive oils, a big variety of plants and fruits, and moderate amounts of wine. This behavior set – or healthy lifestyle – is known as Mediterranean Diet and it is considered an intangible cultural heritage by The United Nations Educational, Scientific and Cultural Organization (UNESCO) since 2010 (UNESCO, 2010).

Besides most known and studied dietary pattern in world, Mediterranean Diet is not the only project of knowledge rescue and ethnobotanic research of native species and foods to increase population’s health. Another example is Ark of Taste project, from Slow Food foundation, which catalogued until now more than 5,000 products that includes native dishes, recipes, species and native products around the world. In Brazil, a term which got famous in the last decade was Unconventional Edible Plants, “Plantas Alimentícias não Convencionais” (PANCs) in Portuguese, which comprises native, regional, weed plants or use of dismissed parts of conventional plants as food (KINUPP, 2007; KINUPP; BARROS, 2008; PEISINO et al., 2019). Besides that, organizations and university, which includes Brazilian Network of Food Data Systems

(Brasilfoods), Universidade of São Paulo (USP) e Food Research Center (FoRC/CEPID/FAPESP) developed a database to list species and food composition from Brazil biodiversity, called “Tabela Brasileira de Composição de Alimentos” – TBCA (http://www.tbca.net.br/). Brazil possesses a huge unexplored biodiversity, but commercial food and agriculture is also based on large domesticated crops of potatoes, rice, beans, and soy. A major exception is cassava, which has processed foods, as tapioca and cassava flour and could be found in most markets. There are also famous regional plants such as “tucupi”, “jambu” and “açaí” at North, “pequi” and “baru nuts” in Midwest, and a huge variety of fruits in Northeast but their production still rarely reaches high scales, commercial exportation or even other parts of Brazil (KINUPP, 2007).

Most studies on wild species are related to nutritional value (MARTINS et al., 2011; RENNA et al., 2015), ethnobotanical surveys (GUARRERA; SAVO, 2016; LENTINI; VENZA, 2007; SHIN et al., 2018), antioxidant and polyphenols quantification (MARTINS et al., 2011; SAVO et al., 2019), but there are a few studies that carried out a detailed chemical profiling of edible species (GIAMBANELLI et al., 2018a, 2018b). Researchers then realized that most of native or wild plants used as food in ethnobotanic surveys were also used by population as phytomedicine to a huge diversity of illness, mainly to inflammatory conditions, fever, gastrointestinal disorders and infections (GUARRERA; SAVO, 2013; LENTINI; VENZA, 2007).

Use of plants as medicine or source of biological active compounds is embedded in humanity’s history. A lot of herbs was used as medicine to treat fever, wounds, gastrointestinal disorders, dermatitis and as painkillers, due to biological activities as analgesia, anti-inflammatory and antimicrobial. Biological activities usually are related to the presence of secondary metabolites, such as phenolics (flavonoids, anthocyanins, cinnamic acid derivatives, etc.), sesquiterpene lactones, coumarins, alkaloids and other classes (HARVEY, 2008; NEWMAN; CRAGG, 2007; WINK, 2003). So, wild plants could be used to combat hunger and malnutrition or used as an alternative to achieve a healthier life, with functional activity and even as prophylaxis to reduce risk and incidence of diseases. On the other hand, still there is a lack of studies about chemical profile, mainly related to secondary metabolites.

Antioxidant and anti-inflammatory activities are the biological activities most studied to edible plants (GUARRERA; SAVO, 2013; MARTINS et al., 2011; SAVO et al., 2019). One possible reason could be that oxidative damage is associated as the first step to more complex conditions, as inflammation cascade triggering, and even development of chronic diseases as cardiovascular diseases, and neurodegenerative diseases as Alzheimer (KARSTENS et al., 2019) and tumorigenesis (VECCHIA, 2009). Antioxidant activity is usually related to the presence of flavonoids or other polyphenols compounds and most of articles in literature rely in just quantitative analysis of presence of flavonoids, total polyphenolic compounds, or antioxidant activity. On the other hand, high biological activity can be found even in extracts with low phenolic contents, which means that chemical composition is more important than phenolic amounts and indicates that profiling the compounds present in each species is necessary to better understand the biological activity (Conforti et al., 2009).

The family Asteraceae

Asteraceae is one of the largest plant families and comprises more than 20,000 species distributed worldwide in more than 1,500 genera. The current family classification is based on morphological and molecular data and comprehend 12 subfamilies and 43 tribes (FUNK et al., 2009).

Taxonomist Funk et al. (2009) describe Asteraceae flower morphology as following: “florets arranged on a receptacle in centripetally developing heads and surrounded by bracts, by anthers fused in a ring with the pollen pushed or brushed out by the style, and by the presence of achenes (cypselas) usually with a pappus”. Asteraceae comprehend herbs, shrubs, trees, and vines, which grow in almost every habitat, with annual or perennial behavior.

Hundreds of Asteraceae species are used as traditional remedies and their biological activities are intensely studied (FUNK et al., 2009). In a previous study, our group investigated the COX and LOX inhibition of 57 Asteraceae species (CHAGAS-PAULA et al., 2015) to demonstrate their anti-inflammatory potential. However, one of the most successful bioactive compounds in Asteraceae is the sesquiterpene artemisinin isolated from *Artemisia annua* L, which was discovered for malaria treatment and as precursor used to obtain more effective derivatives for malaria treatment, which lead

Tu You-You to be awarded with the 2015 Nobel Prize in physiology or medicine (TU, 2011; TU et al., 1982).

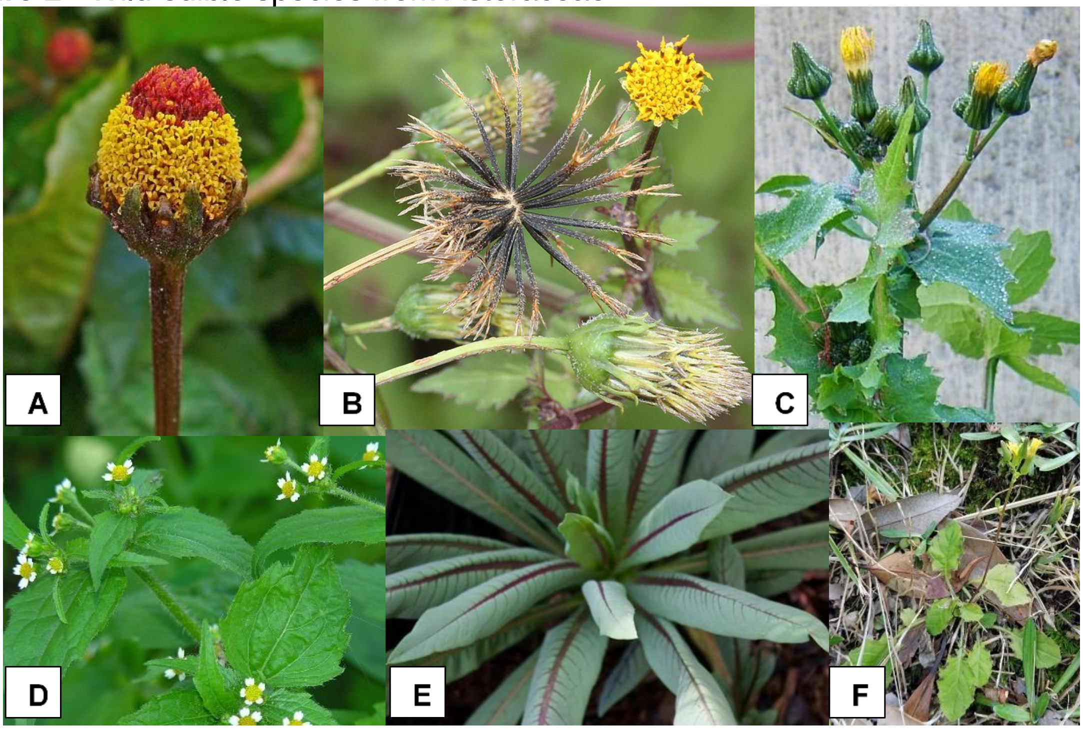
Asteraceae biological diversity influences the chemical composition variety of species. The biosynthesis of secondary metabolites comprehends a broad diversity of compounds in this family, including monoterpenes, diterpenes, triterpenes, sesquiterpenes and sesquiterpene lactones, polyacetylenes, flavonoids, phenolic acids, benzofurans, coumarins and pyrrolizidine alkaloids. Two of these classes, polyacetylenes and special sesquiterpene lactones, are found only in this family (FUNK et al., 2009). Pyrrolizidine alkaloids class, which will be discussed in a separated topic in this work, also is an unusual class restricted to a few plant families and, in Asteraceae, pyrrolizidine alkaloids production is restricted to the tribes Senecioneae and Eupatorieae (NORDENSTAM et al., 2009). The Asteraceae biodiversity associated with chemical diversity makes the family an interesting subject for chemotaxonomy studies (SAREEDENCHAI; ZIDORN, 2010; SHULHA; ZIDORN, 2019).

In opposition to the huge species variety, just a few Asteraceae species are used as domesticated crop. A few of the most known examples are sunflower, lettuce, and chicory (FUNK et al., 2009). Even so, these commodities are among most produced crops in the world. FAO data estimates that production of lettuce and chicory in 2018 in the world was over 42,000,000 tonnes (FAO, 2018). Except for sunflower, which seeds are used as well as for extraction of fixed oil, the edible Asteraceae plants described herein have its leaf used to be eaten, lettuce is eaten raw, most of other are eaten cooked, but raw consumption have also been reported (LORENZI; KINUPP, 2014).

The common lettuce (*Lactuca sativa* L.) is one of the major food commodities in the world and domesticated use dates from ancient Egypt 4,500 BC (HARLAN, 1986). It is considered the domesticated form of the wild species *L. serriola* L. (RYDER; WHITAKER, 1995). Chicory (*Cichorium intybus* L.) is native to Mediterranean region. *Cichorium intybus* L. comprises diverse varieties, such as chicory, Belgian endive, witloof, catalogna type, coffee chicory, and radicchio. *Cichorium endivia* L. have uncertain origin and comprises escarole, with broad-leaves, and endive, with narrow leaves (FUNK et al., 2009). Studies describe lettuce and chicory as good sources of glycosylated flavonoids, caffeic acid derivatives and sesquiterpene lactones, since they can be consumed raw in salads, avoiding degradation of these compounds (PEPE et al., 2015). Sesquiterpene lactones are described as the responsible by bitterness of lettuce and chicory (PRICE et al., 1990). Sesquiterpene lactones shown activity to a few tumoral cell lines, which increased interest in these compounds as edible plants (ZIDORN, 2008).

On the other hand, Asteraceae diversity still could accomplish edible plants if we consider species not investigated, since several ethnobotanical studies reported that the majority of encountered wild edible species belongs to Asteraceae family (GUARRERA; SAVO, 2013; LICATA et al., 2016; RENNA et al., 2015). A few examples of Asteraceae wild edible species used around the world are *Acmella oleracea* (L.) R. K. Jansen, *Bidens pilosa* L., *Sonchus oleraceus* (L.) L., *Galinsoga parviflora* Cav., *Lactuca canadensis* L. and *Youngia japonica* (L.) DC (**Figure 1**).

Figure 1 - Wild edible species from Asteraceae



SOURCE: Wikimedia. Attributions: A) *Zell* B) *Alpsdake* C) Wildfeuer.
A) *A. oleracea*; ***B)*** *B. pilosa;* **C)** *S. oleraceus;* **D)** *G. parviflora;* **E)** *L. canadensis;* **F)** *Y. japonica*

The species *A. oleraceae,* popularly known in Brazil as “jambu”, have flowers and leaves used as condiment in North of Brazil, with presence of spilanthol and N-alkylamides in both parts of the plant (CHENG et al., 2015). *Acmella oleraceae* is used by population as local anesthetic, mainly to toothache, which studies report biological activity to spilanthol, and demonstrated anti-inflammatory and antinociceptive activities, by inhibition of proinflammatory mediators (RONDANELLI et al., 2019; WU et al., 2008).

The species *S. oleraceus* is among the most gathered wild edible plant in Mediterranean area (GUARRERA; SAVO, 2013). In Brazil it is known as “serralha” and its first official report of medicinal use dates from 1874 (RICARDO et al., 2017) as diuretic and to treat nephritis. Crude hydroethanolic (50%) extract of *S. oleraceus* aerial parts demonstrated anti-inflammatory, antipyretic, anxiolytic, and antinociceptive activities in rodent models. The chemical composition reports occurrence of sesquiterpene lactones from the guaianolide and eudesmanolide and mainly derivatives from lactucin (ZIDORN, 2008), and the flavonoids apigenin and luteolin glycosides (SAREEDENCHAI; ZIDORN, 2010).

Bidens pilosa, popularly known as “picão-preto”, has traditional use to several illnesses and has anti-inflammatory, anti-pyretic, and hepatoprotective effects (RICARDO et al., 2017). Phytochemical studies comprehend almost 200 compounds described to this species, with main importance to acetylenes and flavonoids from aurone, chalcone and flavanone classes (SILVA et al., 2011).

The species *G. parviflora,* known as “fazendeiro” or “picão-branco” in Brazil or “guasca” in Colombia, Peru and Bolivia, are traditionally used as anti-inflammatory to treat dermatological problems as eczema and it has in its composition flavonoids, flavanones, caffeic acid derivatives, diterpenes and steroids and high level of vitamin C (ALI; ZAMEER; YAQOOB, 2017; KINUPP, 2007).

Lactuca canadensis, known as “almeirão-roxo” in Brazil, has high levels of minerals Ca, Zn, B and Mn (SILVA et al., 2018). The presence of guaianolides and eudesmanolides sesquiterpene lactones was described previously (MICHALSKA; SZNELER; KISIEL, 2013).

Youngia japonica is distributed throughout the temperate zone and it is used as antipyretic and for detoxification (YAE et al., 2009). Its chemistry comprehends flavonoids, cinnamic acid derivatives, and phenylpropanoids as well as guaianolides and eudesmanolides sesquiterpene lactones (YAE et al., 2009; ZIDORN, 2008).

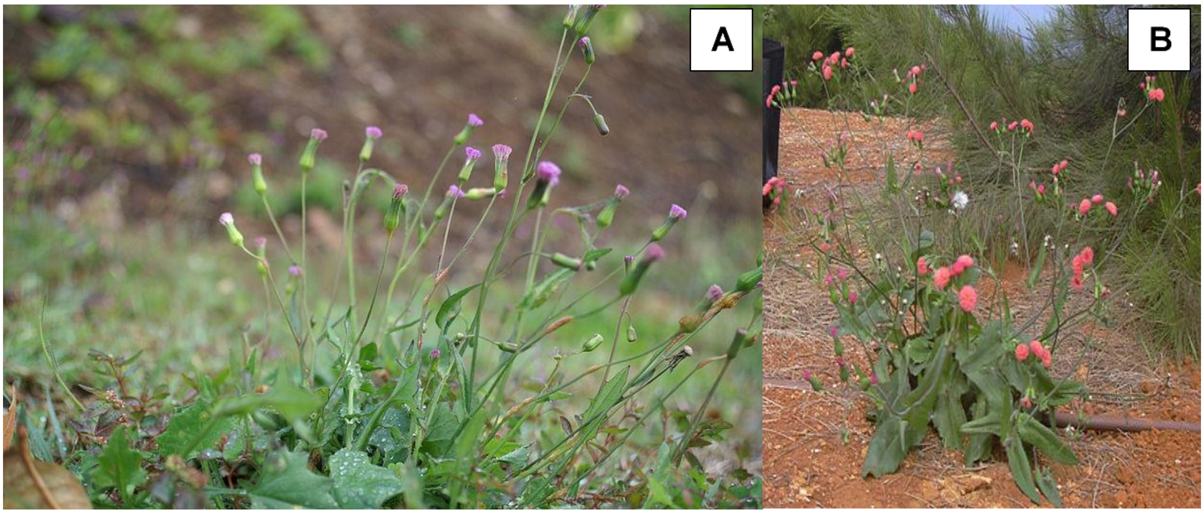
Pyrrolizidine alkaloids and the genus Emilia

Pyrrolizidine alkaloids (PAs) are ester alkaloids composed by a necine base, which can be saturated (platynecine base) or unsaturated (otonecine and retronecine bases). PAs with unsaturation in the necine moiety are transformed into highly reactive pyrroles, which are able to bind to proteins and even DNA cross-links, the cause of genotoxicity potential which could cause tumorigenesis (CHEN; HUO, 2010; MATTOCKS, 1968). Main affected organs in mammals are the liver, where bioactivation occurs, and the lung (CULVENOR et al., 1976). PA poisoning initial symptoms include upper abdominal discomfort, reduction of urine excretion, painful hepatomegaly, and abdominal and feet swelling. The clinical manifestation most frequently caused by PA poisoning is called veno-occlusive disease, which cause obstruction of blood vessels, mainly from liver to heart, which could have fast progression, causing blood vomiting and has high mortality rate. Even after recovery, patients could develop cirrhosis (CULVENOR et al., 1976; MOREIRA; PEREIRA; ANDRADE, 2018; WHO, 1989). The Brazilian Health Regulatory Agency (in Portuguese, “Agência Nacional de Vigilância Sanitária”, ANVISA) restricts PAs maximum daily intake at 1 ppm in phytomedicines or contaminated food (ANVISA, 2014), same restriction limit is reported by MOREIRA & PEREIRA (2018) to the German Health Government. However, the same author cites that Food and Drug Administration (FDA) and Austria government had banned all products containing PAs from markets, since a few vigilance departments consider that no tolerable daily intake could be established.

PAs comprehend a very important information to chemotaxonomy, since PAs are an unusual class and its subclasses pattern is different to each tribe or plant family (HARTMANN; OBER, 2000). PAs are present only in Asteraceae (Eupatorieae and Senecioneae tribes) and Boraginaceae families and a few genera from Fabaceae and Orchidaceae families (HARTMANN; OBER, 2000).

Senecioneae is one of the most diverse tribes in Asteraceae family and comprehend around 150 genera and more than 3,400 species spread worldwide (NORDENSTAM et al., 2009). In Brazil, Senecioneae represents 97 species classified in eight genera. The genus *Emilia* (Cass.) Cass. has currently more than 110 species (The Plant List), however, a global taxonomic review is needed because of the current classification only includes species from Central Africa and the *E. coccinea* complex (JEFFREY, 1997; LISOWSKY, 1990). In Brazil only two species are present: *E. sonchifolia* and *E. fosbergii* (**Figure 2**) (HIND, 1993).

Figure 2 – Aerial parts of *E. sonchifolia* (**A**) and *E. fosbergii* (**B**).



SOURCE: Wikimedia. Attributions: A) Rose; B) Starr.

Misidentification is a serious problem to these *Emilia* species. *Emilia fosbergii* is sometimes misidentified as *E. coccinea* or *E. sonchifolia* var. *javanica*, present only in Oceania and Asia (NICOLSON, 1980). Worldwide known as tassel flower, the most usual popular name used for both species in Brazil is "emília", "serralhinha" or "serralha vermelha". However, both *Emilia* species are sometimes identified in Brazil as "serralha", the popular name of *S. oleraceus*, and even "dente-de-leão", the popular name of *Taraxacum officinale* L.; both these plants are used as phytomedicine, food and/or condiment (KINUPP, 2007; LEITÃO et al., 2014). The misidentification of these species becomes an aggravated problem due to described presence of hepatotoxic PAs in *Emilia* genus.

E. sonchifolia is originated from Asia (NICOLSON, 1980) and used a long time ago in Traditional Chinese Medicine and in Ayurveda Indian medicine to treat a lot of issues such as fever, wounds, respiratory affections, and inflammatory diseases. Some studies confirmed the anti-inflammatory and antinociceptive activities (COUTO et al., 2011; MUKO; OHIRI, 2000). Were also evaluated antitumoral and cytotoxic activity (SHYLESH; PADIKKALA, 2000) with a potential inhibition of metastasis in mice caused by B16F10 melanoma (GEORGE; KUTTAN, 2016). *Emilia sonchifolia* is also used as food by populations from Asian countries, such as Bangladesh, Taiwan and Hong

Kong (GEORGE; KUTTAN, 2016; KUMAR et al., 2015) and even Brazil (KINUPP, 2007).

Previous phytochemical studies of the genus *Emilia* have generally been restricted to *E. coccinea* and *E. sonchifolia*. In these two species, flavonoids, chlorogenic acid derivatives, acetylenes, and ketoesters were described, which could explain the anti-inflammatory medicinal use (BOHLMANN; KNOLL, 1978; RAJ, 2012; SHEN et al., 2013; SRINIVASAN; SUBRAMANIAN, 1980). Most health-related issues and chemotaxonomic significance reports for this genus are associated with the presence of toxic PAs. A total of 11 macrocyclic rings PAs were reported until now in *E. sonchifolia* including N-oxides, retronecine and otonecines bases (HSIEH et al., 2015). The GC-MS analysis of *E. sonchifolia* plant extracts allowed the identification of PAs containing both retronecine (senecionine, seneciphylline, and integerrimine) and otonecine bases (senkirkine, otosenine, neosenkirkine, petasitenine, acetylsenkirkine, acetylpetasitenine, desacetyldoronine, and doronine) (HSIEH et al., 2015). In *E. coccinea* extract, LC-MS analysis detected platyphylline-N-oxide, three stereoisomers of the non-toxic platyphylline, ligularidine, neoligularidine, neosenkirkine, and senkirkine (MROCZEK et al., 2004). Emiline was isolated from *E. flammea*, a synonym of *E. coccinea* (Barbour and Robins, 1987; The Plant List, 2013), and until now, it was described only in this species.

The origin of *E. fosbergii* Nicolson is still undefined, but the main hypothesis regarding its origin suggests that this species is a neotropical descendant of *E. sonchifolia* (MORAES; GUERRA, 2010; NICOLSON, 1980). Peisino et al. 2019 reports the use of *E. fosbergii* as an edible plant with antioxidant and anti-inflammatory properties, probably due to the presence of a few flavonoids and chlorogenic acid. But pyrrolizidine alkaloids were not investigated until now in *E. fosbergii*.

Metabolomics and multivariate analysis

Metabolomics is the youngest in the “omics” research area, which includes genomics, proteomics and transcriptomics (FIEHN, 2002). An acceptable definition to metabolomics is a quantitative or qualitative analysis of a wide number of metabolites, compounds with low molecular weight, from a biological system, as a specific cell, tissue or organism (GOODACRE, 2005; ROCHFORT, 2005). However, there is no strategy able to extract or to analyze the entire range of metabolites present in a biological system yet, since metabolome is composed by a wide range of compound classes with very broad physicochemical properties related to extraction as polarity, solubility and properties directly related to analysis, as UV absorption, fluorescent emission, or mass ionization (GOODACRE et al., 2004; KOPKA et al., 2004; VILLAS-BÔAS; RASMUSSEN; LANE, 2005).

One of the simplest metabolomics subdivisions splits metabolomics in two approaches: targeted and untargeted analysis. Targeted analysis aims to identification and absolute quantification of metabolites selected before analysis. Untargeted analysis also known as metabolite/metabolic profiling is a rapid and qualitative analysis of a large number of different metabolites which aims to identify a specific metabolic profile or trends of metabolites variation that characterize a determined sample, and often absolute quantification it is not necessary (VILLAS-BÔAS et al., 2004).

Metabolomics analysis is possible because of the advance of analytical instruments and machine learning knowledge. The most used techniques to metabolomics are nuclear magnetic resonance (NMR) and mass spectrometry (MS), usually hyphenated or indirectly associated to chromatography. Association between gas or liquid chromatography to mass spectrometry (GS-MS and LC-MS, respectively) is one of the most used technique due to high sensitivity, which allows analysis of metabolites even in very low concentration and comprehensiveness of data, since fragmentation (MS2 or MS/MS) provide structural information and are relatively easy to understanding.

Different methods are necessary to understand the huge amount of information generated by metabolome dataset, for this, multivariate analysis and chemometrics are used to simplify the information and consolidate the knowledge. Chemical measurements obtained by analytical tools, usually called raw data, are treated and analyzed by multivariate analysis to search for patterns or trends able to characterize samples. In unsupervised multivariate analysis, which include principal component analysis (PCA) and hierarchical clustering analysis (HCA), it is possible to observe samples grouping based only in quantitative measurement of data information, which means that samples are grouped by chemical profile similarity (HASTIE; TIBSHIRANI; FRIEDMAN, 2009; VILLAS-BÔAS et al., 2004). PCA is an unconstrained ordination method which aims to represent the main trends of variation of the data (BORCARD; GILLET; LEGENDRE, 2018). In supervised analysis a previous information must be stablished before analysis, for example “active and inactive plant extracts”, and samples information are constrained ordered by these groups sets. Supervised analysis usually needs a training set to build a model, a validation set to ensure quality of the model and a test set, which usually is the set to have information to be analyzed. A few examples of popular supervised analysis tools are partial least square discriminant analysis (PLS-DA) and its orthogonal variety (OPLS-DA), and neural networks (HASTIE; TIBSHIRANI; FRIEDMAN, 2009; VILLAS-BÔAS et al., 2004).

The identification of metabolites is essential to ensure the information and to be integrated by other researches (CREEK et al., 2014; SUMNER et al., 2007). Metabolite identification still is one of the major problems in natural products metabolomics. Secondary metabolism generates outnumbered metabolites from a huge variety of classes and comprehends hundreds or thousands of molecular skeletons (ERNST et al., 2014). In 2007, minimum information to assure identification accuracy level was proposed by the Metabolomics Standard Initiative, where four confidence levels of identification were established (from 1 to 4) (SUMNER et al., 2007). This system was latter adapted (BLAŽENOVI et al., 2018) and the level 0 of identification was added, resulting in the system described in Erro! Fonte de referência não encontrada..

Table 1 - Confidence level of identification.

Confidence level[1]	**Compound [2]**	**Description**
Level 0	Isolated	Isolated compound. Require 3D structure, including stereochemistry.
Level 1	Identified	Standard or reference physical compound match. At least two orthogonal techniques, as MS/MS, or MS and RT.
Level 2	Annotated	Probable structure. Compound matches to literature information at least in two orthogonal information, as MS and MS2 fragmentation.
Level 3	Characterized	Possible structure or class. One or several candidates possible (including isomers), requires at least one information to support propose identification, as MS, MS2 fragmentation or UV profile.
Level 4	Unknown	Unknown feature. Presence in sample.

Adapted from [1] Blaženovi et al. (2018) and [2] SUMNER et al., (2007).

1.1.1 Plant metabolomics

Metabolomics is a crescent field and calls attention of researchers from the areas of natural products and plant research (ERNST et al., 2014). A few applications of metabolomics approaches include drug discovery from medicinal plants, quality control of food and medicinal plants, biogeographic and tracking, environmental metabolomics and chemotaxonomy (CASOTI et al., 2018; PADILLA-GONZÁLEZ; DIAZGRANADOS; COSTA, 2017).

Plant chemotaxonomy can be shortly described as taxonomical organization based on chemical constituents, usually secondary metabolites, in which biosynthetic capacity to produce a set of compounds could be used to distinguish species, genus, tribes or families (SPRING, 2000). It is used as an alternative to understand or as a complementary information to taxonomic classification. Chemotaxonomy is a consistent field in Asteraceae since this wide family have a complex taxonomic classification (DA COSTA; TERFLOTH; GASTEIGER, 2005; SAREEDENCHAI; ZIDORN, 2010; SHULHA; ZIDORN, 2019).

Metabolomics is an approach that can be used to understand the entire plant mechanisms to interact with ambient. Resource availability can influence plant growth rate as well as stressed conditions can influence secondary metabolites biosynthesis and accumulation, that have a wide range of functions in plants (COLEY; BRYANT; CHAPIN, 1985; WINK, 2003). So, plant metabolomics can be used to understand the influence of cultivation, diseases, climates and/or environmental in production of secondary metabolism as well as primary metabolism injures (CASOTI et al., 2018). In a previous study of our research group, it was possible to infer main climate and soil nutrients which induce production of each main class of secondary metabolites from *Tithonia diversifolia* (Hemsl.) A. Gray (SAMPAIO; EbDRADA-EBEL; DA COSTA, 2016).

2 HYPOTHESES

Use of wild species could have vantages and risks. Use of species with presence of toxic compounds or with unknown chemical composition could put population in risk.

On the other hand, the use of wild species could be an alternative to poor population and/or a rich source of bioactive secondary metabolites.

In this context, this study presents two hypotheses:

1) Can *E. fosbergii* be a potential risk to population if used as food?
2) Are there remarkable differences in the chemical profiles of domesticated crops when compared with wild species from Asteraceae used as food?

Objectives

To answer these two main questions, this study has the following specific aims:

1a) to dereplicate leaf extract and investigate the presence of pyrrolizidine alkaloids in *E. fosbergii*;

1b) to compare the metabolic profiles of *E. fosbergii* and *E. sonchifolia;*

1c) to verify the influence of cultivation and flowering in the chemistry profile of *E. fosbergii* and *E. sonchifolia*;

2) to verify differences and similarities between nine edible species of Asteraceae, including three domesticated crops and six wild species.

3 CONCLUSION

In order to have a good health, a balanced diet is recommended, and it can be achieved by increasing the consumption of high amounts of vegetables. It could be done by promoting the collection and/or domestication of wild species as a public health politics measure together with the stimulation of rural economy, bringing together improvement of population's general health and economy growth. However, there is a risk to the population when considering that toxic plants could be ingested, by disinformation or by misidentification.

With regards to the first question, routine consumption of *E. fosbergii* for long periods is a risk to population, since it was found toxic pyrrolizidine alkaloids in this species, including otonecine base, which is considered the most toxic between pyrrolizidine alkaloids. In controlled cultivation experiment, it was possible to see influence of cultivation variables and collect time in amounts of metabolites present in leaves. *Emilia fosbergii* presented higher contents of PAs than *E. sonchifolia*, which increases the risk to population, since there is a misidentification problem in this genus, even with species from other genera. Still, chemical profiles of both species were highly influenced by flowering, which influenced flavonoids and caffeoylquinic acid derivatives accumulation. In *E. fosbergii*, higher contents of PA were found in plants grown in soil with lower contents of nitrate and nitrite, which could increase toxicity.

To answer the second hypothetical question, this study shows a comparison between wild and domesticated edible species from Asteraceae family. Metabolic fingerprint comparison demonstrates chemical difference mainly between species from different tribes. The use of *B. pilosa*, *Y. japonica*, *G. parviflora* and *A. oleracea* as edible plants could bring taste diversity, new sensations, and a variety of secondary metabolites to population. Species *L. canadensis* and *S. oleraceus* had shown similar chemical profile to *C. endivia* and a few samples of *L. sativa*, which could be an alternative to population with no access to domesticated crop species.

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