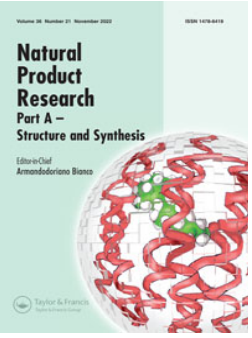

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Antimicrobial and antioxidant properties and quantitative screening of phytochemicals of *Fraxinus excelsior* L. and *Eschscholtzia californica* Cham. mother tinctures

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ABSTRACT

The antioxidant and antimicrobial activities of *Fraxinus excelsior* L. and *Eschscholtzia californica* Cham. mother tinctures against a range of foodborne bacteria were investigated to determine the major components and to analyse the action spectrum and antimicrobial effectiveness of the extracts. Results demonstrated a significant antioxidant activity of *Fraxinus excelsior* L. and a lower activity of *Eschscholtzia californica* Cham. and a good chemical phenolic composition with the highest content of flavonoids. The *Fraxinus excelsior* L. and *Eschscholtzia californica* Cham. mother tinctures demonstrated a middle-high antimicrobial activity against, respectively, 66.67% and 43.33% of all tested bacteria. The inhibitory activity showed a moderate effect on the growth of the sensitive strains in presence of extracts minimum inhibitory concentration. The synergistic actions of bioactive compounds detected in the extracts might be on the basis of antioxidant and biological activities observed and should be used in pharmaceutical, food preservation, alternative medicine and natural therapies fields.

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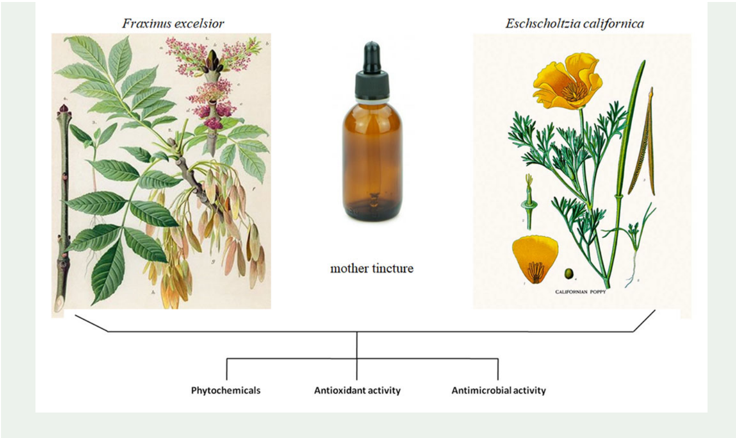

1. Introduction

Plants extracts have been used for a wide variety for many thousands of years for their association with health benefits. In particular, the antimicrobial activity of these extracts is the basis of different applications, such as food preservation, pharmaceuticals, alternative medicine and natural therapies (Sarfraz et al. 2017; Bonomo et al. 2020a). Over the past 20 years, the consumption of natural foods and vegetal supplements/drugs based on natural compounds has significantly increased; food/natural components play an active role in the prevention of chronic diseases or also in the longevity and life quality improvement (Bonomo and Salzano 2012; Mang et al. 2015; Margină et al. 2015; Guidetti et al. 2016). Natural products demonstrated strong antimicrobial potential and they were found to be healthier natural preservatives. The increasing of consumer demand for foods prepared with natural preservatives, prompted companies to use their as alternatives to synthetic compounds, reducing side effects. The economic aspect and the antibacterial efficacy have yet to be resolved in order to fully switch to natural products (Bouarab Chibane et al. 2019).

The beneficial health effects of several plant extracts have been known and studied for centuries and the search for new natural extracts, such as mother tinctures (TMs), to be use in the food and cosmetics industry, is very important at present (Russo et al. 2012; Bonomo et al. 2020b). The TMs contain a variety of specialised metabolites, such as flavonoids, anthocyans, saponins and tannins and their antioxidant capacity promote the use as natural food additives (Bilia et al. 2007; Gutierrez et al. 2008; Laciar et al. 2009).

Fraxinus excelsior L., belonging to the Oleaceae family, is commonly known as ash tree and locally as ‘l’ssane l’ousfour’, in various regions of world (Zulet et al. 2014). Extracts and bioactive metabolites from this plant have been found to possess a variety of biological activities as anticancer, anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, antiallergic, skin regenerating, antirheumatic and diuretic (Kostova and Iossifova 2007; Sarfraz et al. 2017).

The Californian poppy, *Eschscholtzia californica* Cham. (Papaveraceae), is an annual plant originating from California where it colonises coastal dunes and arid areas. Its chemical composition is well known and about 30 tertiary and quaternary isoquinoline alkaloids belonging to six types have been isolated from its various parts (Beck and Haberlein 1999). Various pharmacological studies reported the sedative and anxiolytic actions of extracts of *E. californica* and the absence of toxic effects (Beck and Haberlein 1999; Fabre et al. 2000).

Numerous aromatic, spice, medicinal and other plants were studied for biological activities, however, scientific information on properties of plants that are less used in cuisine and medicine is still rather scarce. Therefore, the assessment of such properties remains a new area for finding sources of natural antioxidants and antimicrobials, functional foods and nutraceuticals (Bonomo et al. 2020a).

The aim of this study was to investigate the antioxidant and the antimicrobial activities of *Fraxinus excelsior* L. and *Eschscholtzia californica* Cham. mother tinctures, against a range of foodborne bacteria, to determine the major components and to analyse the action spectrum and the antimicrobial effectiveness of the extracts.

2. Results and discussion

In the last years, there has been a growing interest in the investigation of biological activities of natural extracts to use in pharmaceutical industries, to avoid side effects on human health (Harvey et al. 2015; Bonomo et al. 2017, 2020b; Sarfraz et al. 2017). A great number of plants showed antioxidant and scavenger activities against free radicals; this antioxidant capacity could be employed in food industry by using plants as a antioxidants source to prevent the rancidity and lipids oxidation (Amamra et al. 2018).

In this study, *Fraxinus excelsior* L. and *Eschscholtzia californica* Cham. mother tinctures were subjected to different assays to screen their antioxidant activity (Table S2). *Fraxinus excelsior* mother tincture reported a significant antioxidant activity. In particular, it showed the highest reducing power determined by FRAP assay with 1972.53 ± 42.01 mg TE/100 mL. *Fraxinus excelsior* was able to scavenge the synthetic DPPH radical showing a value of 418.23 ± 36.11 mg TE/100 mL and it inhibited the lipid peroxidation, reducing the bleaching of β-carotene (A.A. 38%).

Eschscholtzia californica presented lower antioxidant activity than *F. excelsior* with values of 91.12 ± 3.66 mg TE/100 mL and 144.12 ± 10.18 mg TE/100 mL in DPPH and FRAP assays, respectively. The lipid peroxidation inhibition was similar to *Fraxinus excelsior* (Table S2).

Plants rich in antioxidant phenolics represent an important food supplements source, beverages and natural remedies for several ailments, by retarding oxidative degradation of biomolecules and, thereby, by improving the quality and nutritional value of food (Bonomo et al. 2020b). Reactive oxygen species can cause oxidative damages associated with many degenerative diseases, ageing and cancer. Currently, it is very important to discover new sources of safe and inexpensive antioxidants from a nature origin, since some synthetic antioxidants showed potential health risks and toxicity (Bonomo et al. 2020a).

As previously demonstrated (Russo et al. 2012; Bonomo et al. 2020a), a single assay cannot determine the antioxidant activity of a phytocomplex; therefore, three complementary approaches were used to assess the antioxidant potential of the mother tinctures.

The scavenging activities of several natural compounds, such as phenolic compounds, flavonoids or crude mixtures of plants, was widely investigated by DPPH radical system and the effect of antioxidants on DPPH was thought to be due to their hydrogen donating ability (Luximon-Ramma et al. 2002; Gul et al. 2011).

Moreover, the content of metabolites present in the mother tinctures was determined. *Fraxinus excelsior* mother tincture reported a value of 313.88 ± 14.30 mg GAE/100 mL, 631.73 ± 19.67 mg QE/100 mL and 41.53 ± 1.48 mg TAE/100 mL of polyphenols, flavonoids and tannins, respectively (Table S2). Instead, *Eschscholtzia californica* proved a lower chemical phenolic composition; flavonoids were the class of bioactive metabolites with the highest value, 331.54 ± 43.46 mg QE/100 mL, twice lower than *Fraxinus excelsior* flavonoids; *Eschscholtzia californica* tannins and polyphenols of were found to be five and three times lower than *Fraxinus excelsior* (Table S2). The highest content of investigated specialised metabolites in *Fraxinus excelsior* is probably linked to the significant antioxidant activity.

The antioxidant activity of plants, mainly attributed to the presence of active compounds, is well founded. Phenolic compounds have been considered to be powerful antioxidants, are capable of scavenging free radicals and act as reducing agents by their redox properties (Bonomo et al. 2020b). Therefore, in this study, the content of secondary metabolites of the mother tinctures was evaluated and the total polyphenolic content showed a linear relationship with DPPH values, as already observed by other authors (Chew et al. 2008; Malencic et al. 2008; Gul et al. 2011).

Several *in vivo* and *in vitro* studies have been executed to describe medicinal properties of different plants and to investigate the action mechanism. Several data revealed a variety of bioactive medicinal components from different species of *Fraxinus* plant that exhibit various biological activities (Zulet et al. 2014; Sarfraz et al. 2017). Phytochemicals obtained from medicinal plants, herbs, seeds, and fruits have shown promising effects in various fields (Zulet et al. 2014; Sarfraz et al. 2017).

In this study, the phytochemical screening showed the presence of polyphenols, flavonoids and tannins, that are the examples of phenolic components with antioxidant properties. Over the years many studies on total phenolic content had been published underling its importance in the medicinal field (Adeolu et al. 2008; Abdalbasit et al. 2009; Ke-Xue et al. 2011). Esculetin, esculin, fraxin, and fraxetin are some of the pharmacologically active components isolated from different species of *Fraxinus* plant. Esculetin has been extensively used in Chinese herbal medicine due to its vast pharmacological activities such as antioxidant, anticancer, antibacterial, and anti-inflammatory (Montó et al. 2014; Wang et al. 2014; Moulaoui et al. 2015). The effects of *Eschscholtzia californica* result from its chemical composition and in particular the presence of specific alkaloids, such as californidine and eschscholtzine. *Eschscholtzia californica* Cham. contains, also, high amounts of other active components, such as quercitin, protoberberines, benzylisoquinolines, aporphines, benzophenanthridines and protopines (Beck and Haberlein 1999; Fabre et al. 2000; Abdellahet al. 2020).

Moreover, extracts demonstrated an important presence of flavonoids, a group of secondary metabolites with antibacterial, antiviral and radical scavenging capacities.

Tannins are involved in defence mechanism to environmental attack (Bonomo et al. 2020a) and their ability to bind protein and precipitate was exploited to evaluate the tannin content in studied mother tinctures. Studies have demonstrated that, low dosages of tannins in the diet can be beneficial to human health and will create a more astringent feel to the taste, although at higher concentration, they inhibit the digestive enzymes and reduce the bioavailability of iron and B12 vitamin. Tannins have shown potential antiviral, antibacterial and antiparasitic effects (Abdalbasit et al. 2009; Mothana et al. 2010). In the past few years, tannins have also been studied for their potential effects against cancer through different mechanisms (Young and Woodside 2001; Bonomo et al. 2020b).

Moreover, the antimicrobial activity and the MIC of the mother tinctures were evaluated against selected bacterial strains of significant importance for human health by using the agar well diffusion assay. Results showed that the *Fraxinus excelsior* mother tincture demonstrated the antimicrobial activity against 66.67% of all tested bacterial species with a middle-high antimicrobial activity (Table S3).

All Gram-negative bacteria were sensitive with a middle activity (inhibition zone ranging from 11.58 to 14.85 mm) (Table S3). *Fraxinus excelsior* showed a high antimicrobial activity against all species belonging to *Enterococcus* genus, except *Enterococcus hirae* that was sensitive with a middle activity of 14.67 mm inhibition zone.

Eschscholtzia californica mother tincture proved a high antimicrobial activity against 26.66% and a middle activity against 16.67% of all tested bacteria (Table S4). All *Enterococcus* species were sensitive with a middle-high activity (inhibition zone ranging from 14.06 to 20.09 mm). The extract showed a low inhibition only against two bacterial species, *Staphylococcus equorum* (DBPZ0241) and *Listeria innocua*, with 9.72 and 8.67 mm inhibition zone, respectively.

As MIC against susceptible bacterial species (Tables S3 and S4), *Fraxinus excelsior* extract inhibited the Gram-negative bacteria with a concentration ranged from 40 to 70 μg/mL. As Gram-positive bacteria, only *Enterococcus faecium* strain required a high inhibitory concentration (100 μg/mL), while the others resulted differently sensitive to the mother tincture, also among strains belonging to the same species. *Enterococcus durans* and *Enterococcus casseliflavus* were inhibited at a low concentration, of 1 and 10 μg/mL, respectively.

Eschscholtzia californica extract inhibited the half of the susceptible bacteria with a high concentration (80–100 μg/mL), while the 26.67% required an inhibitory concentration of 40–60 μg/mL. *Enterococcus faecium*, *Enterococcus faecalis* and *Enterococcus durans* strains were the most sensitive to the mother tincture with a low inhibitory concentration of 5, 5, and 10 μg/mL, respectively.

Moreover, the efficacy of inhibitory action of *Fraxinus excelsior* and *Eschscholtzia californica* was determined by evaluating bacterial growth by the plate count of those bacterial strains towards which each mother tincture produced a high inhibition zone and showed a MIC of less than 120 μg/mL.

Figure S1 presented the results of the *Fraxinus excelsior* efficacy towards 8 bacterial strains. The inhibitory activity showed a moderate efficacy on the growth of the most of strains in presence of different extract MIC, while only *Staphylococcus equorum*

(DBPZ0044) strain presented a count reduction of nearly 2 log cycles in presence of the mother tincture at 60 μg/mL concentration. Moreover, the inhibitory effect was very low towards *Carnobacterium maltaromaticum* strain in presence of the extract at 40 μg/mL concentration. It's interesting to remark that the lowest concentrations used (1 and 10 μg/mL) showed moderate efficacy with a count reduction of about 1.2 log cycles for *Enterococcus durans* and of over 1.4 log cycles for *Enterococcus casseliflavus*.

Figure S2 presented the results of the *Eschscholtzia californica* efficacy towards five bacterial strains. The inhibitory activity showed a moderate efficacy on the growth of *Enterococcus faecium*, *Enterococcus faecalis* and *Enterococcus durans* strains in presence of very low extract MIC (5, 5, and 10 μg/mL, respectively) with a count reduction of about 1.2–1.4 log cycles. *Brochothrix thermosphacta* and *Staphylococcus equorum* (DBPZ0044) strains proved a count reduction of about 0.8 log cycles in presence of the mother tincture concentrations of 40 and 100 μg/mL, respectively.

These results highlight the antibacterial effects of the mother tinctures that with antioxidant capacity establish a relationship between these activities and the contents of phytochemicals. Plant extracts contain active compounds, which have antioxidant and antibacterial effects and could be useful in the treatment of pathologies where these activities are needed (Beck and Haberlein 1999; Fabre et al. 2000; Amamra et al. 2018).

The inhibitory activity of the extracts is due to presence of polyphenols and flavonoids in large quantity, which have been widely reported as antimicrobial agents (Coppo and Marchese 2014). The antibacterial activity of flavonoids against gram-positive and gram-negative bacteria has been reported (Bylka et al. 2004). The compounds containing hydroxyl groups in ring B, with flavanone aglycones and their derivatives turned out active, demonstrated activity against gram-positive bacteria; while, activity against gram-negative bacilli was demonstrated by the flavones compounds (Kostic et al. 2012).

Plants produce diverse specialised metabolites, such as alkaloids, terpenoids, steroids and polyphenolic compounds, and these phytochemicals are distributed in various parts of the plants and possess important biological properties. Most of these metabolites are significant for plants to prevent herbivores, pathogens and insects, attraction of pollinators and to cope with abiotic stress, etc. They are also known to exhibit several bioactivities, such as antimicrobial, anticancer, antioxidant, and neuroprotective, thus showing a great potential for medicine, industry, agriculture and food sciences. Furthermore, microorganisms indicated a resistance to synthetic antimicrobial agents, which is a serious and immediate concern (Manian et al. 2008, Bonomo et al. 2013; Bonomo et al. 2020b). Due to these facts, the exploration of new alternative medicines derived from plants is required.

3. Conclusions

In conclusion, *Fraxinus excelsior* L. and *Eschscholtzia californica* Cham. mother tinctures displayed antimicrobial and antioxidant potential. The observed antioxidant and biological activities might be due to the synergistic actions of bioactive compounds detected in the mother tinctures. The results of this study could be applied in pharmaceutical field, establishing an important role of mother tinctures in phytotherapy, in order to adopt integrated strategies to effectively counter the excess and the effects of free radicals, and also in food preservation, alternative medicine and natural therapies.

Further studies are needed to elucidate mechanisms that contribute to the extract biological properties and also an in-depth phytochemical investigation is proposed to isolate the active fraction and eventually the pure compound(s) with a vital role for these activities.

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