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# ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES AND PHYTOCHEMICAL SCREENING OF DIFFERENT MOTHER TINCTURES AGAINST FOOD-BORNE BACTERIA

Bonomo M.G.[1*]; Cafaro C.[1]; Russo D.[1]; Faraone I.[1]; Milella L.[1]; Saturnino C.[1]; Capasso A.[2]; Sinicropi M.S.[3]; Salzano G.[1]

[1]*Dipartimento di Scienze, Università degli Studi della Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy;*

[2]*Dipartimento di Farmacia, Università degli Studi di Salerno - Via Giovanni Paolo II 132, 84084 Fisciano (SA), Italy*

[3]*Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, 87036 Arcavacata di Rende, (CS), Italy*

*mariagrazia.bonomo@unibas.it

## Abstract

In this study, the antioxidant and antimicrobial activities of different mother tinctures against a range of food-borne bacteria and their major components were analyzed and determined. The antioxidant results demonstrated a significant activity of different mother tinctures; radical-scavenging activity of the hydroalcoholic extracts were found to be the highest in *Aloe socotrina* and *Crataegus oxyacantha* (330.12±83.22 and 266.71±28.32 mg TE/100 ml, respectively). The ferric reducing power FRAP was 1753.54±224.64 mg TE/100 ml, indicating the highest antioxidant ability of *Aloe socotrina* extract; while, the BCB method evidenced that *Asparagus officinalis, Calendula officinalis* and *Hieracium pilosella* mother tinctures were the most active.

The amount of total phenols ranged from 12.43±0.61 to 944.02±2.53 mg GAE/100 mL, observed in *Asparagus officinalis* and *Aloe socotrina*, respectively. The highest flavonoid content was found in *Aloe socotrina* with 15137,67±1268.90 mg Quercetin/100 mL, while the lowest content was found in *Asparagus officinalis* and *Carduus marianus*. *Aloe socotrina* and *Crataegus oxyacantha* had the highest tannins content, 457,58±2.84 and 342.76±19.73 mg Tannic acid/100mL, respectively; while *Asparagus officinalis* and *Calendula officinalis* showed the lowest value.

The antimicrobial activity and the MIC of the mother tinctures were evaluated against selected bacterial strains; Results provided a different inhibitory effect intra- and inter-family. The most effective extracts were those belonging to Asteraceae family, in particular *Tarassacum dens leonis, Hieracium pilosella, Echinacea angustifolia* and also *Prunus cerasus* of Rosaceae family.

The observed antimicrobial and antioxidant activities might be due to the synergistic actions of bioactive compounds detected in the mother tinctures and could be applied in pharmaceutical field, establishing an important role of mother tinctures in phytotherapy and also in food preservation, alternative medicine and natural therapies.

**Keywords:** *mother tinctures, polyphenols content, flavonoids content, tannins content, antimicrobial activity, MIC, antioxidant properties.*

## Introduction

In the last decades much attention has been dedicated to natural antioxidants and antibacterials and their association with health benefits [1].
Antioxidants are a group of substances that, when present at low concentration in relation to oxidizable substrates, significantly inhibit or delay oxidative processes [2]. Antioxidants are classified into two groups, namely, primary or chain-breaking antioxidants and secondary antioxidants, depending on their mechanism of action. The former react with lipid peroxy radicals to convert them to stable products; this group includes chain breakers (or free radical inhibitors) and peroxide decomposers. Secondary antioxidants, such as oxygen scavengers, reduce the rate of chain initiation. The main characteristic of an antioxidant is its ability to trap free radicals, highly reactive specie having an unpaired electron [3].

The free radicals (FR) and reactive oxygen species (ROS) are produced through frequent physiological and biochemical processes in the human body as byproduct [4]. Over production of such free radicals might leads to oxidative damage of biomolecules in the body (e.g. lipids, proteins, DNA) that can initiate number of diseases like atherosclerosis, diabetes mellitus, cancer, heart and neurodegenerative diseases etc. [5]. The harmful effect of the free radicals can however, be blocked by antioxidant substances. Plants produce wide array of secondary metabolites such as phenolic compounds (phenolic acids, flavonoids, quinines and coumarins), nitrogen compounds (alkaloids and amines), vitamins, terpenoids and other secondary metabolites that have been proved for antioxidant activities [6]. Current research has confirmed that antioxidants are the most effective tools to eliminate free radicals which cause oxidative stress and are possible protective agents that protect from cardiovascular, immune/autoimmune and degenerative diseases [7-9].

Moreover, natural phytochemicals, that can derived from different part of plants, have been reported to possess a wide range of biological activities including also antimicrobial properties [10-15].

The beneficial health effects of extracts from many types of plants have been known for centuries and the search for new natural extracts, such as mother tinctures (TMs), to be used in the food and cosmetics industry, is very important at present. TMs are plant extracts obtained by macerating the fresh herbal drug in the hydroalcoholic solvent in the portion of 1:10 (calculated on the dried weight of the herbal drug), according to the French Pharmacopoeia and their homeopathically diluted solutions are used to treat several ailments [1, 8]. Because of the extraction mode, the hydroalcoholic solvent is able to extract and preserve the whole bioactive substances of plants, so the TMs contain a variety of secondary metabolites, such as flavonoids, anthocyans, saponins and tannins. The antioxidant capacity of these compounds can promote their use as natural food additives [1, 8].

Recently, there has been a growing interest in the investigation and introduction of medicinal plants with various biological activities to the pharmaceutical industries since synthetic drugs have been associated with several side effects on human health. Furthermore, microorganisms indicated a resistance to synthetic antimicrobial agents, which is a serious and immediate concern [16]. Due to these facts, the exploration of new alternative medicines derived from plants is required. Flavonoids are classified under phenolic groups in plants which have been known to possess antimicrobial activity. The mechanisms of flavonoids that are antimicrobial can be classified as the inhibition of nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism [17-19]. Antimicrobial activities of various herbs and spices in plant leaves, flowers, stems, roots, or fruits have been reported by many workers [10, 17, 20]. In contrast, there are no/few reports of antimicrobial activities of hydroalcoholic extracts (mother tinctures) [1, 19, 21].

The potential and targeting of medicinal plants as possible sources of natural antioxidants for food and pharmaceutical products stems from their long-standing use in folk medicine worldwide. The plant kingdom has been the best source of remedies for curing a variety of disease and pain. This is why medicinal plants have played a key role in the worldwide maintenance of health. Natural products of higher plants are an important source of therapeutic agents; therefore, many research

groups are currently screening the different biological activities of plants [12, 22-25].

In the present work, antioxidant and antimicrobial abilities of mother tinctures from twelve medicinal plants were investigated; eight (*Tarassacum dens leonis, Hieracium pilosella, Echinacea angustifolia, Calendula officinalis, Cynara scolymus, Carduus marianus, Arnica montana* and *Arctium lappa*), belonging to Asteraceae family, two (*Prunus cerasus* and *Crataegus oxycantha*) belonging to Rosaceae family and two (*Aloe socotrina* and *Asparagus officinalis*) belonging to Liliaceae family. A mother tincture is an hydroalcoholic preparation obtained from fresh plant or part of plant, by maceration process in ethanol solution, preserving the maximum natural antioxidant levels.

The aim of this study was to evaluate the phenols, flavonoids and tannins content and to investigate and compare the antioxidant and antimicrobial activities of these edible and inedible medicinal plants because they have ethnobotanical value and are used by human beings to treat various ailments.

## Materials and Methods

***Hydroalcoholic extracts***

Hydroalcoholic extract of twelve medicinal plants were purchased by Laboratoires Boiron (France). Extracts were not dried and ethanol was used as solvent at different percentage to extract the active compounds; *Taraxacum dens leonis, Arnica montana* and *Asparagus officinalis* were solubilised with 45% ethanol solution, *Echinacea augustifolia* and *Crataegus oxycantha* with 55% ethanol solution, *Hieracium pilosella, Calendula officinalis*, *Cynara scolymus, Carduus marianus*, *Arctium lappa, Prunus cerasus* and *Aloe socotrina* with 65% ethanol solution.

***In-vitro antioxidant activity***

*2,2-diphenyl-1-picrylhydrazyl (DPPH) test*

Radical-scavenging activity was evaluated by using DPPH test [26]. Briefly, 120 µl of mother tincture or dilutions were added to 1380 µl of 100µM DPPH solution in a microcentrifuge tubes. After 60 min in the dark, the absorbance was monitored at 515 nm by using spectrophotometer (UV 640 Spectrophotometer). Trolox was used as standard and results were expressed as mg Trolox equivalent/100mL of hydroalcoholic extract. Experiments were carried out in triplicate.

*Ferric Reducing Ability Power test (FRAP)*

The reducing power was determined by FRAP assay [26]. The stock solution included 300 mM acetate buffer (3.1 g $C2H_3NaO_2{\cdot}3H_2O$ and 16 ml $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3{\cdot}6H_2O$ solution. The working solution was prepared by mixing acetate buffer, TPTZ and $FeCl_3{\cdot}6H_2O$ (10: 1: 1). Hydroalcoholic extracts (150 µl) were allowed to react with 2850 µl of the FRAP solution for 40 min at 37°C. Readings of the colored product (ferrous tripyridyltriazine complex) were taken at 593 nm. Trolox was used as standard and results were expressed in mg Trolox equivalent /100 ml of hydroalcoholic extract.

*Beta carotene bleaching assay (BCB)*

The antioxidant activity was evaluated by the β-carotene-linoleic acid bleaching method as reported by Russo et al. [8]. β-carotene (0.2 mg) dissolved in 0.2 ml of chloroform, linoleic acid (20 mg) and Tween 20 (200 mg) were mixed. Chloroform was removed by using rotary evaporator at 37°C and distilled water (50 ml) was added. Four milliliters of the emulsion were transferred into several tubes containing 0.2 ml of each mother tinture or ethanol as control. BHT was used as positive control. The tubes were placed at 50°C for 3 h and the absorbance was measured at 470 nm by using spectrometer. The results are expressed as percent of antioxidant activity (%AA).

***Total polyphenolic content***

The content of polyphenols of investigated mother tinctures was measured by using Folin-Ciocalteu assay [26]. Briefly, 50 µl of hydroalcoholic extract and 450 µl of distilled water was added to 500 µl Folin Ciocalteu reagent and 500 µl of $Na_2CO_3$ (10% w/ v). The mixture was mixed and after 1 h of incubation in the dark at room temperature the absorbance was measured at 723 nm using a UV-Vis spectrophotometer (UV 640 Spectrophotometer). The total phenolic content was expressed as mg Gallic Acid Equivalent (GAE)/100 ml of hydroalcoholic extract. The experiment was carried out in triplicate.

***Flavonoid content determination***

The aluminium chloride colorimetric method was used to determine flavonoids content in the sample [2]. Mother tincture (150 µl) was added to 45 µl of 5 % $NaNO_3$ into a microcentrifuge. After 5 minute, 90 µl of 10 % $AlCl_3$ was added. At the $6^{th}$ minute, 300 µl of 1 M NaOH solution and 915 µl of water were added. The solution was mixed well and the absorbance was measured after 10 minutes at 510 nm. Results, carried out in triplicate, were expressed as mg Quercetin Equivalent (QE)/100 ml of hydroalcoholic extract.

***Tannin content determination***

The content of total tannin was measured by protein precipitation assay as reported by Armentano et al. [2]. An aliquot of hydroalcoholic extract (250 µl) was added to 500 µl of BSA solution (1.0 mg/ml in 0.2 M acetic buffer, pH 5.0 with 0.17 M NaCl, buffer A) in a microcentrifuge tube. After 15 min, the solution was centrifuged for 15 min at 5000 g. The surface of the pellet and the walls of the tube were washed with buffer A and the precipitate was dissolved in in 1 ml of SDS-triethanolamine solution. Ferric chloride reagent (1 ml) was added, and the solutions were mixed immediately. The absorbance at 510 nm was taken after 30 min. Results were expressed as mg Tannic Acid Equivalent (TAE)/100 ml of hydroalcoholic extract. Experiments were carried out in triplicate.

***Antibacterial activity assay***

*Bacterial strains and growth conditions*

The mother tinctures were tested *A. hippocastanum* was tested against a panel of bacterial strains shown in table 1. A total of thirty strains of the culture collection of the Dipartimento di Scienze, Università degli Studi della Basilicata, Potenza, Italy, were employed as screening microorganisms for this study. All strains were maintained as freeze-dried stocks in reconstituted (11% w/v) skim milk, containing 0.1% w/v ascorbic acid and routinely cultivated in optimal growth conditions (Table 1). These bacteria were chosen in order to represent the diversity of species of food-borne gram positive and gram negative.

*Agar well diffusion assay and Minimum Inhibitory Concentration*

Antimicrobial activities of each mother tincture were determined by standard agar well diffusion assay [21]. For each strain, a subculture in a specific broth was obtained from the active stock culture by 1% (v/v) inoculum and incubated overnight at the corresponding culture temperature. 200 µl of each subculture was used to inoculate the agar media (to achieve a final concentration of $10^6$ CFU/ml) and distributed into Petri plates. 60 µl of the extract was poured into wells (6 mm diameter) bored in the agar plates and then the plates were incubated at optimal growth conditions for each strain. Organic solvent was used as negative control while chloramphenicol antibiotic was used as positive control. The experiment was performed in triplicate and the antimicrobial activity of the extracts was expressed in terms of zone of inhibition diameter mean (in mm) produced by the respective extract after 24 h of incubation. An inhibition zone <9 mm indicated a low antimicrobial activity; 10< zone of inhibition <14 mm, a middle antimicrobial activity; a zone of inhibition >15 mm, an high antimicrobial activity. Then, extracts were screened to determine minimum inhibitory concentrations (MICs) in order to evaluate the antimicrobial effectiveness of extract against different bacterial strains by the agar well diffusion method [21]. Each specific medium inoculated with the strain subculture was distributed into Petri plates and different concentrations of extract, ranging from 1 µg/ml to 120 µg/ml, were poured into wells bored in the agar plates and the plates were incubated for 24 h. After incubation, the MIC was calculated as the lowest concentration of each extract inhibiting the growth of bacterial strains. The MIC values were done in triplicate.

## Results and Discussion

Plants produce diverse types of metabolites, some produced during secondary metabolism and referred to as secondary metabolites. Important among these are alkaloids, terpenoids, steroids and polyphenolic compounds. These chemicals (phytochemicals) are distributed in various parts of the plants. The exact role of these metabolites is not fully understood, however, most of these metabolites are of significance for plants in terms of preventing herbivores, pathogens and insects, attraction of pollinators, coping with abiotic stress etc. [3, 27]. Besides, these phytochemicals are known to exhibit a range of bioactivities such as antimicrobial, anticancer, antioxidant, antiherbivore

and insect repellent activity. These plant secondary metabolites have a great potential for medicine, industry, agriculture and food sciences. It is of profound importance to detect the phytochemicals in plants so as to relate their presence with bioactivity observed and to know their possible therapeutic role [21, 28]. Plants rich in antioxidant phenolics represent an important source of food supplements, beverages and natural remedies for several ailments, by retarding oxidative degradation of biomolecules and, thereby, by improving the quality and nutritional value of food [29, 30]. Reactive oxygen species can cause oxidative damages associated with many degenerative diseases such as atherosclerosis, dementia, inflammation, coronary heart diseases, ageing and cancer. Currently, it is very important to discover new sources of safe and inexpensive antioxidants from nature origin, since some synthetic antioxidants showed potential health risks and toxicity [8].

In this study, the hydroalcoholic extracts were subjected to the 2,2-diphenyl-1-picrylhydrazyl (DPPH), β-carotene bleaching (BCB) and ferric reducing antioxidant power (FRAP) assays to screen their antioxidant activity (Table 2). As previously demonstrated [8], 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 antioxidant results demonstrated a significant activity of different mother tinctures; the Trolox equivalent activity per 100 ml of mother tincture (mg TE/100 ml), measured by DPPH test, showed an high radical-scavenging potential of 330.12±83.22 and 266.71±28.32 mg TE/100 ml, in *Aloe socotrina* and *Crataegus oxyacantha* respectively; while, *Hieracium pilosella* and *Arnica montana* proved a middle-high radical-scavenging value, 182.21±5.14 and 156.26±30.22 mg TE/100 ml, respectively. *Asparagus officinalis* extract had the lowest antioxidant activity with 1.73±0.95 22 mg TE/100 ml.

The antioxidant activity of extracts is usually associated to their reducing capacity, therefore, the FRAP assay was a reliable method to study the antioxidant activity of various compounds and to evaluate rapidly the total antioxidant capacity of different plant extracts containing flavonoids [7, 31]. As shown in Table 2, the ferric reducing power FRAP was 1753.54±224.64 mg TE/100 ml indicating the highest antioxidant ability of *Aloe socotrina* extract. *Asparagus officinalis* showed the lowest reducing power (1.76±0.04 mg TE/100 ml). Moreover, the BCB assay inhibit the Beta carotene bleaching at 50% when tested. 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 [7, 31]. The lipid peroxidation inhibitory activity of the extracts by the β-carotene bleaching test was assessed and the results are shown in Table 2. The oxidation of β-carotene and linoleic acid generates free radicals. The linoleic acid free radical formed upon the abstraction of a hydrogen atom attacks the highly unsaturated β-carotene molecule, hence β-carotene is oxidized, losing its orange color which is then monitored spectrophotometrically. In the absence of an antioxidant β-Carotene undergoes rapid discoloration.

Results were expressed as percentage of antioxidant activity (%AA) and they showed that *Asparagus officinalis* (53,73±7.56%), *Calendula officinalis* (49,50±16.34%) and *Hieracium pilosella* (48,59±8.77%) mother tinctures were the most active. The other extracts showed a middle activity.

Over the past few years, investigations for phenolics compounds in medicinal herbs have gained importance. It is well-known that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma, and flavor and also in providing health-beneficial effects [28, 29]. They are secondary metabolites synthesized by plant directly involved in the response of the plants to different type of stress; they contribute to healing by lignifications of damaged areas, they possess antimicrobial and antioxidant activity and their concentration may increase after infection [26].

The antioxidant activity of extracts, 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 [21].

For this reason, in this study, the content of secondary metabolites of the mother tinctures was also evaluated by *in vitro* assays (Table 2).

The total polyphenolic content of the mother tinctures was determined by Folin-Ciocalteu method. All extracts proved a good total polyphenolic content, showing a linear relationship with DPPH values, as already observed by other authors [7, 32, 33].

The amount of total phenols (Table 2) ranged from 12.43±0.61 to 944.02±2.53 mg GAE/100 mL of hydroalcoholic extract, values observed in *Asparagus officinalis* and *Aloe socotrina*, respectively.

Each mother tincture was, also, tested to evaluate flavonoid and tannin content by using two different colorimetric assays, aluminium chloride and protein precipitation methods, respectively. Several studies reported the ability of flavonoids to attract pollinators to flowers and animals to fruits, in order to disperse the seeds of the plant, and, also, they described their significant antioxidant and chelating properties. Antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups [31]. The highest flavonoid content was found in *Aloe socotrina* with 15137,67±1268.90 mg Quercetin/100 mL of extract, much more higher than the mean value. The lowest content was found in *Asparagus officinalis* with 134,09±42.65 and in *Carduus marianus* with 160.22±22.60 mg Quercetin/100mL. Data are shown in Table 2.

Tannins are involved in defence mechanism to environmental attack [21] 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 [22, 33]. In the past few years tannins have also been studied for their potential effects against cancer through different mechanisms [4, 14, 15]. In this study, data revealed that *Aloe socotrina* and *Crataegus oxyacantha* had the highest tannins content, 457,58±2.84 and 342.76±19.73 mg Tannic acid/100mL of extract, respectively; while *Asparagus officinalis* and *Calendula officinalis* showed the lowest value in tannin content with only 5.27±0.76 and 7.09±0.86 mg Tannic acid/100mL of extract, respectively.

Moreover, the antimicrobial activity and the MIC were evaluated against selected bacterial strains of significant importance for human health, by using the agar well diffusion assay. A total of thirty gram-negative and gram-positive bacteria were employed as screening microorganisms to determine the antimicrobial effect and the action spectrum of each hydroalcoholic extract. Results showed that mother tinctures demonstrated antimicrobial activity against some tested bacterial species, providing a different inhibitory effect intra- and inter-family. The most effective extracts were those belonging to Asteraceae family, in particular *Tarassacum dens leonis, Hieracium pilosella, Echinacea angustifolia* and also *Prunus cerasus* of Rosaceae family; these mother tinctures, even though they didn't show antimicrobial activity against half of tested bacteria (from 40.6% to 59.4%), they proved an high inhibitory effect on 21.9-25% of strains and middle-low on 18.7-24%, except for *Hieracium pilosella* with a middle-low activity on 40.6% of bacterial strains. The other Asteraceae family extracts and the further Rosaceae family tincture exhibited a rather feeble inhibitory activity, that was low or absent against the most of strains and high only on some strains of Enterococcus genus. As Liliaceae family extracts, *Asparagus officinalis* showed the same behaviour of the latter described, while *Aloe socotrina* was the less effective tincture with no antimicrobial activity against 90.6% of strains and only middle-low effect on 9.4% (data not shown). All tested bacteria are foodborne, the most of them comes from meat/naturally fermented meat products and the others from milk/milk products. Of these, some strains are food spoilage bacteria, other strains are selected autochthonous starter cultures and others are foodborne type strains.

*Escherichia coli* was sensitive only to *Hieracium pilosella* and *Echinacea augustifolia* extracts that showed a low antimicrobial activity with 9.77 and 8.83 mm diameter inhibition zone, respectively. The most effective extracts (*Tarassacum dens leonis, Hieracium pilosella, Echinacea angustifolia* and *Prunus cerasus*) inhibited the growth of all food spoilage bacteria; they showed an high inhibitor activity

against *Brochothrix thermosphacta* and a middle-low effect on *Pseudomonas fragi*, while all of these, except for *Tarassacum dens leonis* proved a middle-low activity on *Hafnia alvei* and *Pseudomonas proteamaculans* (data not shown).

All extracts were found effective against *Enterococcus* spp. strains with a middle-high antimicrobial activity (inhibition zone ranging from 12.78 to 18.32 mm), while the inhibition of *Weissella* spp. strains was different according to species tested. *Weissella minore* and *Weissella confusa* were sensitive to the extracts with a middle-high and middle-low antimicrobial activity, respectively, while for the other strains no inhibition zone was observed. Moreover, among *Staphylococcus* spp. strains, the extracts had a middle-high antimicrobial effect on *Staphylococcus succinus* and a *Staphylococcus equorum* strain, while *Staphylococcus xylosus* and the other *Staphylococcus equorum* strains showed a low or absent sensitivity, except for one of the latter strains that showed a high sensitivity to *Prunus cerasus*. Finally, none of the extracts exhibited the antimicrobial effect on *Lactobacillus* strains tested (data not shown).

The active extracts with an high antimicrobial activity were subjected to determine MIC by the agar well diffusion method against respective susceptible bacterial species (Table 3). *Tarassacum dens leonis* tincture mother showed a MIC of 40 µg/ml for *Carnobacterium maltaromaticum*, *Brochothrix thermosphacta* and *Staphylococcus Staphylococcus equorum*, while two strains belonging to *Enterococcus* species required an inhibitory concentration of 10 µg/ml and the other two >120 µg/ml. As *Hieracium pilosella* tincture, *Enterococcus faecalis* and *Enterococcus casseliflavus* strains were the most sensitive with a MIC of 5 µg/ml, while the other strains, resulted sensitive to this extract, required an inhibitory concentration of 40 µg/ml (Table 3). *Echinacea augustifolia* extract showed a MIC of 1 µg/ml against *Enterococcus faecalis* and *Enterococcus durans* and an inhibitory concentration of 10 µg/ml against the other sensitive *Enterococcus* spp. strains; *Staphylococcus succinus* required a concentration of 100 µg/ml, while the other sensitive strains of 40 µg/ml (Table 3).

As the other five tinctures belonging to Asteraceae family, the strains resulted sensitive were inhibited only by the substance itself, except for *Enterococcus faecalis* that was sensitive to 20 µg/ml of *Calendula officinalis*, *Cynara scolymus* and *Carduus marianus*, and *Enterococcus durans* to 20 µg/ml of *Arctium lappa*. As the extracts of Rosaceae family, *Prunus cerasus* was the only tincture with an high antimicrobial activity against a *Staphylococcus equorum* strain and *Weissella minore*, with an inhibitory concentration of 40 µg/ml and >120 µg/ml, respectively. The two sensitive *Enterococcus* strains required a MIC of 10 µg/ml, while the other strains a MIC ranging from 40 to 100 µg/ml. *Crataegus oxycantha* extract inhibited *Enterococcus faecalis* and *Enterococcus durans* strains with a concentration of 20 µg/ml and 10 µg/ml, respectively, while the strain of *Carnobacterium maltaromaticum* only as substance itself (Table 3).

Finally, the Liliaceae family extracts were the least active and *Asparagus officinalis* was able to inhibit the *Enterococcus* spp. strains resulted sensitive only as substance itself (Table 3).

The antibacterial activity of flavonoids against both Gram-positive and Gram-negative bacteria has been reported [34]. 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 [35].

In conclusion, the different 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 contributes to these extract 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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| Bacterial species | Growth conditions | |
|---|---|---|
| | Temperature | Medium |
| *Carnobacterium maltaromaticum* | 20°C | Tryptone Soya Yeast Extract Medium |
| *Carnobacterium divergens* | 20°C | Tryptone Soya Yeast Extract Medium |
| *Pseudomonas fragi* | 20°C | Tryptone Soya Yeast Extract Medium |
| *Hafnia alvei* | 30°C | Tryptone Soya Yeast Extract Medium |
| *Pseudomonas proteamaculans* | 30°C | Tryptone Soya Yeast Extract Medium |
| *Brochothrix thermosphacta* | 20°C | Tryptone Soya Yeast Extract Medium |
| *Escherichia coli* | 37°C | Tryptone Soya Yeast Extract Medium |
| *Enterococcus hirae* | 37°C | M17 Medium |
| *Enterococcus faecium* | 37°C | M17 Medium |
| *Enterococcus faecalis* | 37°C | M17 Medium |
| *Enterococcus casseliflavus* | 37°C | M17 Medium |
| *Enterococcus durans* | 37°C | M17 Medium |
| *Enterococcus gallinarum* | 37°C | M17 Medium |
| *Weissella viridescens* | 30°C | MRS Medium |
| *Weissella confusa* | 30°C | MRS Medium |
| *Weissella hellenica* | 30°C | MRS Medium |
| *Weissella minore* | 30°C | MRS Medium |
| *Weissella cibaria* | 30°C | MRS Medium |
| *Weissella paramesenteroides* | 30°C | MRS Medium |
| *Lactobacillus sakei* | 30°C | MRS Medium |
| *Lactobacillus sakei* | 30°C | MRS Medium |
| *Lactobacillus sakei* | 30°C | MRS Medium |
| *Lactobacillus sakei* | 30°C | MRS Medium |
| *Lactobacillus sakei* | 30°C | MRS Medium |
| *Staphylococcus xylosus* | 30°C | Tryptone Soya Yeast Extract Medium |
| *Staphylococcus equorum* | 30°C | Tryptone Soya Yeast Extract Medium |
| *Staphylococcus equorum* | 30°C | Tryptone Soya Yeast Extract Medium |
| *Staphylococcus succinus* | 30°C | Tryptone Soya Yeast Extract Medium |
| *Staphylococcus equorum* | 30°C | Tryptone Soya Yeast Extract Medium |
| *Listeria innocua* | 30°C | Tryptone Soya Yeast Extract Medium |

**Table 1.** Bacterial strains and growth conditions used for antimicrobial activity assay.

| Mother Tinctures | Family | % EtOH | BCB | DPPH | FRAP | Total phenols content | Total Tannins content | Total flavonoids content |
|---|---|---|---|---|---|---|---|---|
| | | | %AA | mgTE/100mL | mgTE/100mL | mgGAE/100mL | mgTAE/100ml | mgQE/100mL |
| ***Arctium lappa*** | Asteraceae | 65 | 16.23±5.23 | 114.59±26.56 | 132.74±28.72 | 63.00±2.69 | 17.31±1.15 | 213.06±14.90 |
| ***Arnica montana*** | Asteraceae | 45 | 39.52±6.73 | 156.26±30.22 | 231.83±46.91 | 103.40±2.34 | 81.63±1.20 | 324.39±13.87 |
| ***Calendula officinalis*** | Asteraceae | 65 | 49.50±16.34 | 46.27±7.35 | 116.01±12.01 | 91.54±6.51 | 7.09±0.86 | 473.14±40.68 |
| ***Carduus marianus*** | Asteraceae | 65 | 29.49±2.92 | 31.62±2.03 | 128.10±13.63 | 118.02±2.42 | 33.45±1.76 | 160.22±22.60 |
| ***Cynara scolymus*** | Asteraceae | 65 | 36.11±4.23 | 91.40±15.74 | 281.98±16.19 | 128.05±1.44 | 58.84±2.03 | 454.63±40.81 |
| ***Echinacea angustifolia*** | Asteraceae | 55 | 25.33±2.83 | 131.33±14.26 | 281.07±36.87 | 123.27±4.06 | 23.01±0.89 | 408.94±65.29 |
| ***Hieracium pilosella*** | Asteraceae | 65 | 48.59±8.77 | 182.21±5.14 | 520.88±23.17 | 186.10±7.82 | 26.07±0.90 | 481.44±59.30 |
| ***Tarassacum dens leonis*** | Asteraceae | 45 | 34.08±9.32 | 48.47±5.38 | 73.27±17.49 | 58.76±4.44 | 24.01±0.45 | 292.63±18.09 |
| ***Aloe socotrina*** | Liliaceae | 65 | 37.08±2.89 | 330.12±83.22 | 1753.54±224.64 | 944.02±2.53 | 457.58±2.84 | 15137.67±1268.90 |
| ***Asparagus officinalis*** | Liliaceae | 45 | 53.73±7.56 | 1.73±0.95 | 1.76±0.04 | 12.43±0.61 | 5.27±0.76 | 134.09±42.65 |
| ***Prunus cerasus*** | Rosaceae | 65 | 36.65±2.29 | 70.29±4.25 | 153.61±12.88 | 72.85±6.32 | 59.63±4.17 | 367.18±12.10 |
| ***Crataegus oxyacantha*** | Rosaceae | 55 | 35.25±6.72 | 266.71±28.32 | 402.85±28.08 | 161.25±4.05 | 342.76±19.73 | 982.17±123.04 |

Values are the mean of three determinations ±Standard Deviation

**Table 2**. Antioxidant activity (BCB, DPPH, FRAP assays) and chemical phenolic composition of mother tinctures

| Bacterial species | MIC (µg/ml) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Taraxacum dens leonis* | *Hieracium pilosella* | *Echinacea angustifolia* | *Calendula officinalis* | *Cynara scolymus* | *Carduus marianus* | *Arnica montana* | *Arctium lappa* | *Prunus cerasus* | *Crataegus oxyacantha* | *Aloe socotrina* | *Asparagus officinalis* |
| *Carnobacterium maltaromaticum* | 40 ± 0.23 | 40 ± 0.34 | 40 ± 0.54 | | | | t.q. | | 40 ± 0.93 | t.q. | | |
| *Brochothrix thermosphacta* | 40 ± 1.03 | 40 ± 0.45 | 40 ± 0.88 | | | | | | 40 ± 0.98 | | | |
| *Enterococcus hirae* | >120 | | | | | | | | | | | |
| *Enterococcus faecium* | | | 10 ± 0.98 | t.q. | t.q. | t.q. | t.q. | t.q. | 10 ± 0.56 | | | t.q. |
| *Enterococcus faecalis* | 10 ± 0.76 | 5 ± 0.31 | 1 ± 0.09 | 20 ± 0.15 | 20 ± 0.67 | 20 ± 0.76 | t.q. | t.q. | 10 ± 0.77 | 20 ± 0.91 | | t.q. |
| *Enterococcus casseliflavus* | | 5 ± 0.28 | 10 ± 0.66 | t.q. | | | t.q. | | | | | |
| *Enterococcus durans* | 10 ± 0.58 | | 1 ± 0.03 | | | | t.q. | 20 ± 0.57 | | 10 ± 0.03 | | t.q. |
| *Enterococcus gallinarum* | >120 | 40 ± 0.77 | | t.q. | | t.q. | | t.q. | | | | |
| *Weissella minore* | | | | | | | | | >120 | | | |
| *Staphylococcus equorum* | | | | | | | | | 40 ± 0.81 | | | |
| *Staphylococcus equorum* | 40 ± 1.22 | 40 ± 0.38 | 40 ± 0.77 | | | | | | | | | |
| *Staphylococcus succinus* | | | 100 ± 1.23 | | | | | | 100 ± 1.12 | | | |

Different concentrations of extracts were tested (from 1 µg/ml to 120 µg/ml) by the agar well diffusion method (Russo et al., 2012).
The values are expressed as mean ± standard deviation

**Table 3**. Minimum inhibitory concentration MIC (µg/ml) of antimicrobial activity of mother tincture


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