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A Study on Antioxidant and Antimicrobial Activity of *Ferulago galbanifera* Species

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Abstract

In this work, antimicrobial effects of the root, stem, leaf and flower parts extracts with ethanol and acetone of *Ferulago galbanifera* species against *Escherichia coli*, *Staphylococcus epidermidis, Enterococcus faecalis Candida albicans, Bacillus subtilis, Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhimurium* strains were investigated by using the disc diffusion method. In addition to that, antioxidant activity of *F. galbanifera* ethanolic extracts was measured using the DPPH method, as well as their total phenolic content using the Folin-Ciocalteu's phenol reagent technique. To our results, ethanolic extracts of leaf from *F. galbanifera* were found to have antimicrobial effect against the all microorganisms, whereas the acetonic extracts of leaf has shown antimicrobial effects to some microorganisms other than *Staphylococcus epidermidis, E. faecalis, C. albicans* species. Extracts obtained from the root and the flower parts of the plant had no antimicrobial effect on the test microorganisms. The antioxidant activity level was found to be in the following order (from the highest to the lowest): flower, leaf, stem and rood; 16.32, 215, 244, 323(µg/mL), respectively. The highest total phenolic content obtained from the parts of the plant *F. galbanifere* was for the root, while the lowest was for its leaf part.

Key Words: *Ferulago galbanifera*, Antibacterial, Antioxidant

1. Introduction

Genus of *Ferulago* W. Koch (Apiaceae) is represented by 34 species, amongst them 19 are endemic in Turkey (Saya et. al. 2012). Since the roots and fruits of the *Ferulago* species contain secretion agent out of essential oil, resin and gum, they have a distinct odour. Their usage and contents are similar to those of *Ferula* species known publicly as the same name and they (*Ferulago* species) are being used as aphrodishiac, tonic and digestive agents as well as to heal hemoroids and ascarids in folk medicine (Akalın 1999; Başer et. al. 2002). *Ferulago* is also used as a spice especially in salads for its specail smell. *Ferulago* species are named as "çaksırotu", "kisnis", "asaotu", "kuzu bası" and "kuzu kemirdi" in various regions of Turkey. The essential oils of secondary metabolits are encountered in the plants growing in the hot climatic regions of the world. Although these so called 'essence' and 'etheric oils' named owing to their smells are present in 100 families' species comprising the one third of the world flora, they are especially seen in some families of Coniferaea, Rutaceae, Apiaceae, Myrtaceae and Labiatae, mainly in their specific tissues

(Graikou et. al. 2012;Mammadov 2014; Jara – Bermeo et. al. 2016).

Essential oils (EOs) are known to exhibit antibiotic and anticeptic effects. With this special characteristics, they are called as antimicrobial agents since they are the alternatives to the food originated pathogens, chemical preservatives and antibiotics and they can destroy bacteria, fungia and yeast (Kürekçi and Sakin 2017).

Antioxidant compounds have the effect of delaying or inhibiting the oxidation of lipids and other parameters. The association of the myriad of compounds present in essential oil provides higher antioxidant activity than the summed activity of the individual components. Essential oils may also be used as food preserving agents owing to the presence of phenolic compounds as main components, which are liable to the antioxidant properties and may be an alternative to synthetic antioxidants (Marin et. al. 2016).

2. Material and Methods

2.1. Materials

For the current study, all parts (root, stem, leaf and flower) of *Ferulago galbanifera* (Mill.) W. Koch plant were collected. The collection area was Alpagut neighbourhood, Mihalgazi town (Eskişehir). After collection the samples were cleaned and dried for 7 days and Some of them were made into herbarium material (M. Sağıroğlu 6568 SAÜ Biology Herbarium).

All the chemicals and reagents (Folin-Ciocalteu, Gallic Acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Methanol, Mueller Hinton Agar (MH agar), Triptic Soy Broth, Sodium Carbonate, Ascorbic Acid) used in this study were of analytical grade and obtained from Merck Company, Germany.

2.2. Preparation of Extracts

Fifteen grams of dried parts (roots, stem,leaf and flower) of the plants were ground into a capped bottle and 150 mL of ethanol and acetone was added on top of those. These prepared extracts are kept in a cool and dark environment for 3 days and mixed in a magnetic stirrer at regular intervals. The solvents in the extracts were evaporated by using a rotary evaporator (Heidolph) under vacuum at 55°C for 15 minutes and the dried extracts were then used for all investigations. The extract concentrations were adjusted by adding own solvent (ethanol or acetone) to each extract at the doses of 6400 µg/disc for the antimicrobial activity tests. Ethanolic exracts were setting 1000µg/ml for the antioxidant activity and the total phenolics analyses. For antimicrobial activity, 15 µL of empty sterile discs with a radius of 6 mm from the raw extracts obtained were absorbed and kept in a dark sterile environment for 24 hours.

2.3. Disc diffusion method

All strains used throughout this study have been obtained from Microbiology Research Laboratory of Sakarya University. The disc diffusion method was used to determine the antimicrobial activities of the extracts. Suspensions with a density of 0.5 McFarland from previously activated microorganisms were prepared by a densitometer. Prepared microorganism suspensions to Müeller Hinton Agar were inoculated with sterile swab. Discs impregnated with extracts were slightly pressed on the inoculated plates under aseptic conditions, followed by an incubation at 37˚C for 24 h. Ethanol and acetone impregnated discs were used as negative controls and the commercial antibiotic discs (Gentamicin and Amphotericin B) were used as positive ones. If there is an inhibition zone against that pathogen around the disc as a result of the incubation, the zone diameters (mm) are

measured from the rear of the petri by using a digital caliper.

2.4. Antioxidant activity (DPPH assay)

The modified Blois method was used for determination antioxidant activity (Blois, 1958). In short, 1 ml of 0.004% solution of DPPH radical in methanol was mixed with 1 mL of extract solution in methanol (containing different concentrations of dried extract). These solutions were kept in dark place for 30 mins and the optical density was then measured at 517 nm using a spectrophotometer and methanol was used for the blank. The following equation was employed to evaluate the % DPPH radical scavenging activity: %DPPH radical scavenging = [(control absorbance- extract absorbance)/control absorbance] x 100.

2.5. Total phenolic content (TPC)

Total phenolic substance determination was determined using modified Folin-Ciocalteu method of Singleton and Rossi (1965). Taking 100 µL of the prepared extract, 200 µL of 50% Folin-Ciocalteu reagent was added and left for 2 minutes. We have then added 1 mL of 2% Na_2CO_3 solution on it and waited for 1 hour in the dark and the absorbance at 760 nm was read. The total phenolic content was determined in mg / 100g using Gallic Acid Standard.

3. Results and Discussion

The polyphenolic constituents that are present in the plant extracts produce various biologic activities together with antioxidant abilities. Nowadays, several studies are focused upon the potential healt benefits of polyphenols and their pharmacological potential as antidiabetic (Asgar, 2013), anticancerogenic (Rosa et al., 2016), antimicrobial and antioxidant (Semerci et al., 2020) agents (Unuofin et al., 2017). In the current study, the extracts obtained from the root, stem, leaf and flower parts of *F. galbanifera* has been worked out for their phenolic contents and the results were given in Table 1. The highest total phenolic content has been observed at the root part, whereas the lowest was at the leaf of the plant. The amount of the content were as follows: root 611.6, flower 311.6, stem 176.6 and leaf 43.4 mgGA/100g.

In vitro antioxidant tests are designed to imitate the oxidation-reduction reaction that are prevalently present in live biological systems and to evaluate the antioxidant potential of various chemical and biological substances (Ebrahimabadi et al., 2010). In this study a common DPPH test to measure the antioxidant activity has been employed. IC_{50} values of the extract obtained and the standard (value needed for scavenging the DPPH 50%) are given in Table1. It has been determined that the highest antioxidant activity level is for flower ethanolic extracts (16.3 µg/mL). When compared with the ascorbic acid standard, it has been found that the flower extract exhibits higher level of antioxidant activity. To our best of knowledge, there is no any study indicating that the plant *F. galbanifera* has antioxidant activity.

Table 1. Antioxidant activity and total phenolic content of the samples

Samples	Antioxidant Activity IC_{50} (µg/mL)±SD	Total Phenolic Content (mgGA/100g)±SD
Flower	16.32±0.28	311.6±2.5
Stem	244±0.8	176.7±8.7
Root	323.6±2.4	611.6±7.75
Leaf	215±1.4	43.4±1.8
Ascorbic Acid	3.2±00.1	_

Table 2. Antimicrobial activity of different parts of *Ferulago galbanifera*

Samples 6400µg/disc		Test microorganism (inhibision zone diameters, mm±SD)							
		Ec	**Se**	**Ef**	**Bs**	**Pa**	**Sa**	**St**	**Ca**
Leaf	Acetone	10.5±0.5	0	0	8.5±0.5	8±0	12.5±05	9.5±0.5	0
	Ethanol	13±0	10.5±0.5	9±0	9±0	8±0	16±0	13±0	8,5±0.5
Stem	Acetone	0	0	0	0	0	8.5±0.5	0	0
	Ethanol	0	0	0	0	0	8	0	0
Flower	Acetone	0	0	0	0	0	0	0	0
	Ethanol	0	0	0	0	0	0	0	0
Root	Acetone	0	0	0	0	0	0	0	0
	Ethanol	0	0	0	0	0	0	0	0
Antibiotic	GC	17	20	19	22	20	21	21	0
	Amp	0	0	0	0	0	0	0	16

Ec: *Escherichia coli,* ***Se:*** *Staphylococcus epidermidis,* ***Ef:*** *Enterococcus faecalis,* ***Ca:*** *Candida albicans,* ***Bs:*** *Bacillus subtilis,* ***Pa:*** *Pseudomonas aeruginosa,* ***Sa:*** *Staphylococcus aureus,* ***St:*** *Salmonella typhimurium,* ***Gc:*** *Gentamicin,* ***Amp:*** *Amphotericin B.*

Mileski et al. (2015) studied the antimicrobial adn antioxidant activities of endemic species of *F. macedonica*. They have concluded that the IC_{50} value of the extract obtained with ethanol was 1100 µg/mL. Golfakhrabadi et. al. (2016) worked out the antioxidant/antimicrobial activity of *Ferulago carduchorum* plant and found that the extract obtained for the flower part with methanol has IC_{50} value of 1 mg/mL, whereas it was 9.4 mg/mL when compared with hexan. It has been deduced that the solvent used in the extract preparation process does affect the antioxidant activity level.

In the current study it has been determined that the prepared leaf ethanolic extract has the IC_{50} value of 215 µg/mL. The difference between the works in the literature and the current one is thought to be originated from the extract preparation techniques and the plant collection areas, as well as the differences in species worked on.

The antioxidative and antimicrobial properties of essential oils obtained from several plants have taken great interest in academic studies and in food, cosmetics and pharmaceutics industries since they are thought to be the natural dopant substance candidates to substitute the synthetic antimicrobial agents. In that work, the antimicrobial activity effect of the extracts obtained from *F. galbanifera* plant *(*with acetone and ethanol) on *Escherichia coli* (ATCC 25922), *Staphylococcus epidermidis* (ATCC 12228), *Enterococcus faecalis* (ATCC 29212), *Candida albicans* (ATCC 10291), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 29213), *Salmonella typhimurium* (ATCC 14028) strains has been

summarized in Table 2. The highest antimicrobial activity has been detected for the leaf extract (with ethanol) on the bacteria *S. aureus*, producing 16 mm inhibition diameter. Ethanolic extract is found to have higher antimicrobial activity level than acetonic extract one.

Demirci et al. (2000) studied the antimicrobial effects of the essential oils of various Ferulago species and found that the highest antibacterial effect of *F. galbanifere* essential oil was detected to be on *E. coli*, followed by *S. aureus*, *S. typhimurium* bacteria. In another work, the chemical constituents and antimicrobial activities of essential oils obtained from the root, stem, leaf and flower parts of *F. trifida* were investigated and concluded that the flower part has produced 18 mm inhibition zone on *S. aureus,* whereas it was measured to be 34, 25 and 20 mm for the stem, the leaf and the root part, respectively (Tavakoli et al., 2017). In the current work, the highest antibacterial activity has been detected to be in *S. aureus* bacteria, though small differences between the parts of the plant. These antimicrobial activity differences in the various parts of the plants is thought to be originated from the fact that the chemical constituents differs in different part of the plants. Shivering the different parts of the plants in the preparation process of the extracts may create distinctive effects. In that work and the literature support this fact (Shaid Ud-Daula et al., 2016; Asraf et al., 2018).

4. Conclusion

The flower part of *Ferulago galbanifera* extracts show strong antioxidant activity due to its distinct content. From the current work together with the similar studies in the literature we conclude that different parts, i.e., flower, stem and leaf, of *F. galbanifera* are suitable to be used as a natural antioxidant and some (leaf part) as antibacterial source. The extract prepared from the leaf was determined to have a significant antibacterial effect on *S. aureus*. In this context, *F. galbanifera* leaves can contribute to natural hand and surface disinfectants and can be used as a food preservative

Conflict of Interest

The authors declare that they have no conflict of interest.

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