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The Evaluation of Antioxidant and Antimicrobial Activities of Essential Oil and Aqueous, Methanol, Ethanol, Ethyl Acetate and Acetone Extract of *Hypericum Scabrum*

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***Abstract*—**Herbal essential oil and extracts are a good source of natural antioxidants and antimicrobial compounds. *Hypericum* is one of the potential sources of these compounds. In this study, the antioxidant and antimicrobial activity of essential oil and aqueous, methanol, ethanol, ethyl acetate and acetone extract of *Hypericum scabrum* was assessed. Flowers of *Hypericum scabrum* were collected from the surrounding mountains of Hamadan province and after drying in the shade, the essential oil of the plant was extracted by Clevenger and water, methanol, ethanol, ethyl acetate and acetone extract was obtained by maceration method. Essential oil compounds were identified using the GC-Mass. The Folin-Ciocalteau and aluminum chloride ($AlCl_3$) colorimetric method was used to measure the amount of phenolic acid and flavonoids, respectively. Antioxidant activity was evaluated using DPPH and FRAP. The minimum inhibitory concentration (MIC) and the minimum bacterial/fungicide concentration (MBC/MFC) of essential oil and extracts were evaluated against *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, Salmonella typhimurium, Aspergillus flavus and Candida albicans*. The essential oil yield of was 0.35%, the lowest and highest extract yield was related to ethyl acetate and water extract. The most component of essential oil was α-Pinene (46.35%). The methanol extracts had the highest phenolic acid (95.65 ± 4.72 µg galic acid equivalent/g dry plant) and flavonoids (25.39 ± 2.73 µg quercetin equivalent/g dry plant). The percentage of DPPH radical inhibition showed positive correlation with concentrations of essential oil or extract. The methanol and ethanol extract had the highest DDPH radical inhibitory. Essential oil and extracts of *Hypericum* had antimicrobial activity against the microorganisms studied in this research. The MIC and MBC values for essential oils were in the range of 25-25.6 and 25-50 µg/mL, respectively. For the extracts, these values were 1.5625-100 and 3.125-100 µg/mL, respectively. Methanol extracts had the highest antimicrobial activity. Essential oil and extract of *Hypericum scabrum,* especially methanol extract, have proper antimicrobial and antioxidant activity, and it can be used to control the oxidation and inhibit the growth of pathogenic and spoilage microorganisms. In addition, it can be used as a substitute for synthetic antioxidant and antimicrobial compounds.

***Keywords*—**Antimicrobial, antioxidant, extract, *hypericum.*

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I. Introduction

MICROBIAL spoilage and oxidation are two unpleasant events during the production, processing, storage and preparation of food products. The lipid phase of the foodstuff is extremely vulnerable to oxidation, especially when it contains a high content of unsaturated fatty acid. After oxidation of fatty acids, peroxide compounds, and subsequently aldehyde and ketone compounds create a sharp, irritating smell. In this phenomenon, lipid compound radicals might react with other macromolecules and therefore convert them to radical. In general, oxidation causes degradation of unsaturated fatty acids and some vitamins, and decreases foodstuff nutritional value; although, the effects of free radicals in the diet are higher than that aforementioned. Many chronic diseases such as cardiovascular and cancer are caused by free radical and the imbalance of antioxidant compounds and oxidant compounds or oxidative stress [1]-[3]. Antioxidants are used to reduce the oxidation process or delay it and have natural or synthetic source. In some studies, toxicity of synthetic antioxidants has become a concern [2]. So, the attention of researchers and the food industry has been attracted to natural antioxidants. These compounds not only lack some of the harmful effects of synthetic antioxidants, but their consumption can lead to more health and wellbeing.

In addition to oxidation, one of the important issues is controlling the growth of microorganisms in food products. Many pathogens are foodborne. In addition to thermal treatment and cooling, chemical preservatives are also used to reduce and eliminate the growth of microorganisms, and to improve the health of the food and increase the shelf-life. Today, many studies are being done to reduce the utilization of synthetic preservatives and their substitution with the natural preservatives.

Herbal essential oil and extracts are a good source of antioxidant and antimicrobial compounds [4]. Essential oils and extracts of different herbs have been studied for antioxidant compounds and antimicrobial compounds. *Hypericum* flower is known as one of the sources of antioxidant and antimicrobial compounds and is widely consumed in the United States and grows in many regions in Asia, Europe and North Africa. It has anti-depressant, antiviral, and anti-cancer properties and is used to treat mild depression and to treat behavioral abnormalities as well as

gastrointestinal disorders [5]. *Hypericum* has 460 species. Several studies have been carried out on *Hypericum.* The results indicate that the chemical composition, the antioxidant and antimicrobial activity of *Hypericum* flower depends on the species, the growth place, the climatic conditions, the genetic characteristics, the method of extraction of essential oil or extract and the time of harvesting [6], [7]. So far, no research on this plant has been conducted in western regions of Iran. The purpose of this study was to evaluate the antioxidant and antimicrobial activity of essential oil and aqueous, methanol, ethanol, ethyl acetate, and acetone extract of *Hypericum scabrum.*

II. Material and Methods

A. Preparation of Extract

Hypericum scabrum flowers are collected from the surrounding mountains of Hamedan. Confirming of species was carried out by assistance of experts in the Research Center of the Jihad-e-Agriculture Organization of Hamadan province, Iran. After drying the flowers in the shade at room temperature for several days, they are powdered. The extract was obtained by soaking in solvents including water, methanol, ethanol, ethyl acetate or acetone. For extracting, 100 g of *Hypericum scabrum* powder is transferred to the Erlenmeyer, and 500 mL of mentioned solvents are added to the powder and the Erlenmeyer lid is closed with parafilm. Erlenmeyer stayed on the shaker for 24 hours, then it is filtered and solvent was added to remained powder and the extraction was repeated for other two times. The extract is concentrated by rotary evaporator at 40 °C and dried with the freeze-dryer, and stored in a container in the freezer at -18 °C.

B. Essential Oil Preparation

Essential oil is prepared using clevenger. For this purpose, 30 g of *Hypericum scabrum* flower powder and are poured into a clevenger flask and 300 mL of water was added. The extraction was done for 3 hours (100 °C) [8].

C. Determination of Phenolic Acid Compounds

Folin-Ciocalteau method is used to measure the amount of phenolic acid. Gallic acid is used as a standard and its various concentration was prepared and its absorption measured at 765 nm by the spectrophotometer. After plotting the calibration curve, the amount of phenolic acid in samples expressed in µg galic acid equivalent/ g dry plant. 0.5 mL of extract or essential oil was mixed with 2.5 ml Folin-Ciocalteau (0.2 N). After 5 minutes, 2 mL of sodium carbonate solution (7.5%) was added and 3o min stored at room temperature. The absorption was measured with a spectrophotometer at 765 nm [9].

D. Determination of Flavonoids

Flavonoids measurement was done by using a spectrophotometer and in accordance with the previous studies [20]. In a test tube, 0.5 mL of the extract/ essential oil was mixed with 25.2 mL of distilled water, 0.15 mL of 5% sodium nitrate and 0.3 mL of 10% $AlCl_3.6H_2O$ solution. After 5 minutes, one mL sodium hydroxide (one molar) was added and mixed. Then, the absorbance is determined by using a spectrophotometer. The calibration curve is plotted by using various concentrations of quercetin. Flavonoids are expressed in mg quercetin equivalent /g dry plant [9].

E. Evaluation of Antioxidant Activity of Extract/ Essential Oil by DPPH Method

50 µL of different concentrations of 20, 40, 60, 80, and 100 µg/mL of extract/essential oil are added to 5 mL of DPPH solution (0.004%) in methanol. After 30 minutes of incubation at room temperature, the absorbance of the samples is read against the blank at 517 nm by using spec photometer. Percentage of inhibition of DPPH free radicals was determined by using: $I\% = (A_{blank} - A_{sample} / A_{blank}) \times 100$. In this formula, A_{blank} shows a negative control absorbance without an extract, and A_{sample} expresses the absorbance of various concentrations of the extract [4].

F. Determination of Antioxidant Activity by FRAP

150 µL extract or essential oil was mixed with 2850 µL of FRAP indicator and after stirring for 15 minutes at room temperature and the absorbance intensity was measured by spectrophotometer at 593 nm. The calibration curve was prepared by concentrations of 125, 250, 500, 750, and 1000 µmolar of $FeSO_4$, $7H_2O$. Antioxidant activity was expressed based on micromole of Fe^{2+}/g dry plant [5].

G. Determination of MIC and MBC/MFC

The impact of Hypericum scabrum essential oil or extract on four types of bacteria (Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa and Salmonella typhimurium) and one type of mold (Aspergillus flavus) and one type of yeast species (Candida albicans) was investigated. MIC and MIB/MFC determination was done by using microdilution method [10].

H. Essential Oil Compounds Analysis

Essential oil compounds of *Hypericum scabrum* are identified using the Agilent GC-Mass device (Santa Clara, United States) [5].

I. Data Analysis

SPSS version 16: 0 software was used to analyze the data. One-way ANOVA and Tukey's post hoc test were used to determine difference among groups. $P < 0.05$ was considered the significance difference.

III. Results and Discussion

The yield amount of *Hypericum scabrum* essential oil was 0.35%. The lowest and highest extracts were obtained by using of ethyl acetate and water solvent (Table I).

In Table II, the chemical compounds of the essential oils of *Hypericum scabrum* analyzed by the GC/MS were shown. The most component of essential oil were α-Pinene (46.35%), 3-methyl-β-Pinene (12.28%) and β-Caryophyllene (4.21%), respectively.

TABLE I
YIELD OF ESSENTIAL OIL AND AQUEOUS, METHANOL, ETHANOL, ETHYL ACETATE AND ACETONE EXTRACT OF HYPERICUM SCABRUM

Essential oil or type of extract	Yield (percentage)
essential oil	0.35±0.04[d]
aqueous extract	12.95±1.02[a]
methanol extract	10.67±1.09[b]
ethanol extract	10.35±1.38[b]
ethyl acetate extract	9.02±1.15[c]
acetone extract	9.52±1.89[c]

Means with different letters in a column are statistically significant (P<0.05).

The methanol extract contained the highest phenolic acid content (95.65 ± 4.72 g µg galic acid equivalent/**g** dry plant) and flavonoids (25.39 ± 2.73 µg quercetin equivalent/**g** dry plant), whereas the aqueous extract had the lowest phenolic acid (41.4 ± 3.14 µg galic acid equivalent/**g** dry plant) and flavonoid (14.19 ± 1.05 µg quercetin equivalent/**g** dry plant) (Table III).

With increasing concentrations of essential oil or extract, the percentage of DPPH radical inhibition was increased. Significant differences (P< 0.05) were observed between the antioxidant activity of extracts and essential oil. The methanol and ethanol extract had the highest inhibition of DDPH radical. *Hypericum scabrum* essential oil has higher antioxidant activity than the aqueous, ethyl acetate, and acetone extract (Fig. 1).

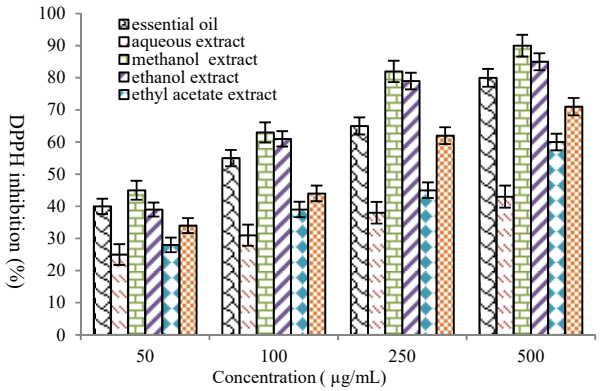

Fig. 1 The antioxidant activity of essential oil and aqueous, methanol, ethanol, ethyl acetate and acetone extract of *Hypericum scabrum* by DPPH assay

The amount of antioxidant activity in IC50 is shown in Fig. 2. Essential oil and extract of *Hypericum scabrum* had antimicrobial activity against the microorganisms studied in this study (Table IV). The MIC and MBC values for essential oils were in the range of 25-6.25 and 6.25-50 µg/mL, respectively. For extracts, these values were 1.5625-100 and 3.125-100 µg/mL. Methanol extracts had the highest antimicrobial activity.

In this study, the essential oil extraction yield was 0.35%. Among the extracts, the highest and lowest extract yields were related to water (12.95%) and acetone (9.02%) extract.

The amount of essential oil yield in this study (0.35%) was higher than results reported for essential oil of *H. helianthemoides* (0.12%), *H. scabrum* (0.20%) and *H. perforatum* (0.21%) collected from Northern Iran (8), *H. scabrum* (0.05%), *H. dogonbadanicum* (0.10%), *H. helianthemoides* (0.06%) collected from southern Iran (Fars province) (9). *Hypericum* species are plants with low essential oil (less than 1%) [11].

TABLE II
THE COMPOSITION OF *HYPERICUM SCABRUM* ESSENTIAL OIL

Chemicals identified	Content (%)	Chemicals identified	Content (%)
Undecane, 5-methyl	0.12	Ylangene	0.1
α-Thujan	1.78	α-Copaene	0.1
α-Pinene	46.35	β-Bourbonene	2.24
α-Fenchene	2.45	β-Cubebene	0.25
Camphene	0.4	β-Elemene	0.1
Nonane, 3-methyl-β-Pinene	8.12	α-Cedrene	0.45
β-Myrcene	4.11	β-Caryophyllene	4.21
α-Phellandrene	1.65	Aromadendrene	1.32
α-Terpinene	1.23	α-Humulen	0.22
p-Cymene	0.9	β-Farnesene	1.27
Limonene	0.35	Bicyclosesquiphellandrene	0.1
β-Phellandrene	1.22	1-Dodecanol	1.02
1,8-Cineole	1.45	α-Amorphene	0.21
(Z) β-Ocimene	0.21	Germacrene-D	1.42
(E) β-Ocimene	0.09	Bicyclogermacrene	0.28
γ-Terpinene	0.05	β-Selinene	1.52
Decane, 2-methyl	1.02	α-Selinene	0.12
Terpinolene	0.09	α-Farnesene	0.45
Undecane	1.22	γ-Cadinene	0.45
n-Nonanal	0	Δ-Cadinene	0.25
1-Octanol, 2-methyl-	0.31	1,4-Cadinadiene	0.25
Fenchol, endo	0.25	Spathulenol	0
α-Campholene aldehyde	0.1	Caryophyllene oxide	0.24
Borneol	0.54	Germacrene	0.35
Terpin 4 ol	0.24	Hinesol	0
Pulegone	0.25	α-Cadinol	0.14
Thymol	0.98	β-Eudesmol	0.1
Tridecane	1.01	α-Eudesmol	0.2
Carvacrol	0.32	Phytol	0
α-Cubebene	0	undefined components	5.83

The most abundant compounds identified in the essential oil were α-Pinene (46.35%), 3-methyl-β-Pinene (12.28%) and β-Caryophyllene (4.21%). Javidnia et al. (2008) found the most important of component of *H. scabrum* essential oil is α-Pinene (59.3%) [12]. Ghasemi Pirbalouti et al. (2014) identified 96% of the compounds in the *H. scabrum* essential oil, which included 31 compounds. The most important components were α-pinene (49.96%), β-pinene (9.70%), limonene (6/6%), ¬ (E) -β-ocimene (5.6%) and carvacrol (5.8%) [8].

Dadkhah et al. (2014) found the most important compounds of *Hypericum* essential oils of Qazvin's Alamut area weree α-pinene (40.9%), spathulenol (7.9%), β-pinene (5.2%) α-cadinol (7.4%), Limonene (3.4%) and epi-α-muurolol (2.3%) [12].

TABLE III
THE AMOUNT OF PHENOLIC ACID AND FLAVONOID COMPOUNDS OF ESSENTIAL OIL AND AQUEOUS, METHANOL, ETHANOL, ETHYL ACETATE AND ACETONE EXTRACT OF *HYPERICUM SCABRUM*

Essential oil or type of extract	Phenolic acid (µg gallic acid equivalent/g dry plant)	Flavonoids (µg quercetin equivalent/g dry plant)
essential oil	75.35±4.31^{d}	18.09±2.10^{bc}
aqueous extract	41.1±3.14^{f}	14.19±1.05^{d}
methanol extract	95.65±4.72^{a}	25.39±2.73^{a}
ethanol extract	90.08±3.05^{b}	24.05±1.91^{a}
ethyl acetate extract	53.21±1.98^{e}	16.35±0.98^{c}
acetone extract	85.41±3.01^{c}	21.05±1.62^{b}

Means with different letters in a column are statistically significant ($P<0.05$).

Many factors, including light, duration of the day, night temperature, flowering stage, affect the biosynthesis of the essential oil and compounds. In addition, the conditions used to extract essential oils, such as temperature, pressure and extraction time, affect the yield or recovery of the extracted compounds [10]-[13]. It seemed that the cause of the difference in the compounds found in *Hypericum scabrum* investigated in our study with the compounds reported in other studies is related to the difference in weather conditions in western Iran, including Hamadan with other places.

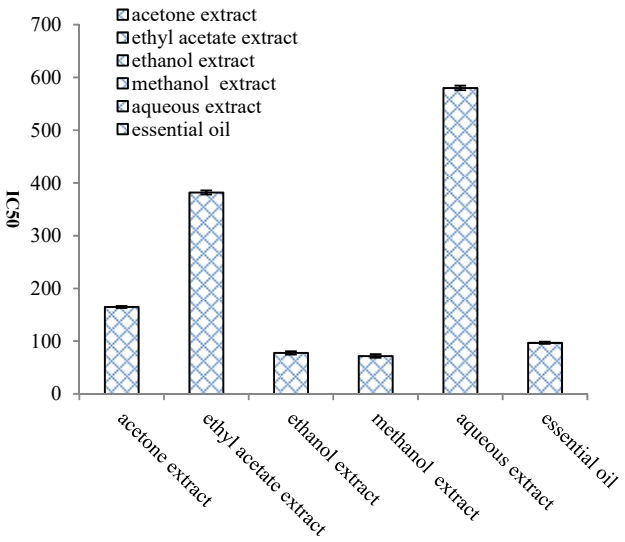

Fig. 2 Antioxidant activity in IC50

TABLE IV
DETERMINATION OF MIC AND MBC/MFC ESSENTIAL OIL AND AQUEOUS, METHANOL, ETHANOL, ETHYL ACETATE AND ACETONE EXTRACT OF *HYPERICUM SCABRUM* ON MICROORGANISMS (µG /ML)

Essential oil or type of extract	*Staphylococcus aureus*		*Bacillus cereus*		*Salmonella typhimurium*		*Pseudomonas aeruginosa*		*Aspergillus flavus*		*Candida albicans*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC	MIC	MFC
essential oil	6.25	12.5	6.25	6.25	25	25	25	50	12.5	25	12.5	25
aqueous extract	50	100	25	50	50	100	100	200	25	50	25	50
methanol extract	6.25	12.5	3.125	6.25	12.5	25	25	25	1.5625	3.125	1.5625	3.125
ethanol extract	6.25	12.5	3.125	6.25	25	25	25	50	1.5625	3.125	1.5625	3.125
ethyl acetate extract	25	50	12.5	12.5	25	50	50	100	3.125	6.25	12.5	12.5
acetone extract	25	50	6.25	12.5	25	50	50	50	3.125	6.25	12.5	12.5

Dadkhah et al. reported a flavonoid amount of 30.8 mg quercetin equivalent/g of aqueous extract [12]. This amount is greater than the value found in the present study (maximum flavonoid content in methanol extract was 25.39 ± 2.73 µg quercetin equivalent/g dry plant).

The maximum amount of phenolic acid in this study (95.65 ± 4.72 µg galic acid equivalent/g dry plant) was lower than that reported in other studies. In a study by Morshedlo et al., the amount of phenolic acid compounds of methanol extract of *H. perforatum* collected from Tonekabon region 50 mg galic acid equivalent/g was reported [5]. Fathi and Ebrahimzade found that the amount of phenolic acid in the methanol extract of *H. perforatum* collected from the sari area was 505.75 mg gallic acid equivalent/g [13]. Other species of Hypericum including *H. caprifoliatum*, *H. carinatum*, *myrianthum* and *H. polyanthemum* had higher antioxidant activity than the results of this study.

The amount of MIC and MBC obtained in this study is less than that reported in the previous studies, which shows the higher antimicrobial activity of *Hypericum scabrum* essential oil and extract of the western region of Iran. Ghasemi Pirbalouti et al. identified the antimicrobial activity of the *H. scabrum* essential oil. The MIC and MBC values obtained for *Bacillus cereus*, *Pseudomonas aeruginosa* and *Salmonella typhimurium* were 125, 250, and 250 µg /mL, respectively [8].

The antimicrobial properties of *Hypericum scabrum* are attributed to the presence of a-pinene. The antimicrobial effect of this compound has been proven [7]. Lipophilic compounds such as terpenoid derivatives have a destructive effect on the membranes of the bacteria and fungi, preventing ion transport and cellular respiration [12]. However, the mechanism of antimicrobial activity of the essential oil and plant extract is very diverse.

According to previous studies, *Hypericum scabrum* has a greater effect on gram positive bacteria than gram negative and methanol extract has stronger antimicrobial properties than aqueous extract [7].

IV. CONCLUSION

Use of essential oil and extract of *Hypericum scabrum* especially methanol extract has an antimicrobial and antioxidant effect and can be used to control the oxidation and inhibit the growth of pathogenic microorganisms in order to replace synthetic antimicrobial and antioxidant compounds.

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