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Research Article

ANTIOXIDANT AND ANTIBACTERIAL EFFECTS OF THE ESSENTIAL OIL AND VARIOUS EXTRACTS OF *DIONYSIA REVOLUTA BOISS*

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ABSTRACT

Objective*: Dionysia revoluta* Boiss. is used in Iranian traditional medicine in gastric disorders and for wound healing. There are no studies for this plant in the literature. We aimed to study the chemical composition of the essential oil of the plant and assess the antioxidant and antibacterial effects of its essential oil and different extracts of this plant. Materials and methods: Essential oil was provided by steam distillation and analyzed with gas/chromatography and mass spectrometry. Essential oil, and various extracts were evaluated for antioxidant effect using diphenylpicrylhydrazil (DPPH) assay. Disk diffusion was used for antibacterial studies. Results and conclusion: GC/MS analysis indicated the presence of acetophenone (29.94%0), acetylphenol (20.23%), and benzaldehyde (14.26%) as main compounds of essential oil. Ethanolic extract showed the highest inhibition DPPH radical with IC_{50} = 98.8µg/ml in comparison to BHT (IC_{50} = 47.19µg/ml). Ethanolic and ethylacetate extracts showed inhibitory effect against the major of tested bacteria except *E. aerogenes* and *C. freundii.* Our results indicated that the ethanolic extract of this plant would be a good candidate for further studies. The essential oil of this plant also exhibited notable antioxidant effect which needs to be studied using the other methods to have valid results.

Keywords: *Dionysia revoluta*; Essential oil; Extract; DPPH; Disk diffusion.

INTRODUCTION

The role of oxidant agents has been known in pathogenesis of many disease such as diabetes, cancer, cardiovascular disease[1]. Essential oils are aromatic secondary metabolites of medicinal plants which as well as flavonoids and phenolics show different activities such as antimicrobial, antioxidant, anticonvulsant, anti inflammatory, diuretic[2-6]. The essential oils as well as phenols and flavonoids can act as antioxidants and antibacterial and have an important role in to prevention of different disorders which are associated with oxidative factors or bacterial agents[4,7]. Considering the problems with synthetic antioxidant and also the side effects and tolerance to common antibacterial drugs, there are many interests in finding new therapeutic agents especially from natural sources.

The genus of *Dionysia* belongs to Primulaceae family and comprises more than 27 perennial specific species in Iran which restrict distributed in dry mountains. Some of these species also have been found in Iraq, Anatolia, Pakistan, Tadzikestan and Afghanistan[8]. The plant of *Dionysia Revoluta* (Boiss.) subspecies *revoluta* is one of these endemic plants which has widely distribution in Iran. This plant is used in gastric disturbance and disinfection of wounds[9]. Previous studies have revealed the antibacterial effect of this plant against of *Staphylococcos aureus* in comparison to Erythromycin and Cephalexin[9]. There are no study for chemical composition and antioxidant and antibacterial effects of this plant in the literature. In continuing the study of antioxidant and antibacterial effects of the extracts and essential oil of medicinal plants, we have studied this plant essential oil and different extracts for evaluation of these biological activities.

MATERIALS AND METHODS

Plant materials

The plant was gathered from the mountains of Sarikhani in Buanat in Fars Province at April, 2011. The plant was dried in shade and was confirmed by Dr. Mirtadzadini, Bahonar University. A voucher specimen was deposited in Herbarium Center of Bahonar University.

Isolation of essential oil

The amount of 200g of dried aerial parts of the plant was subjected to hydrodistillation for 3 h. using cleavenger apparatus to yield 0.75% essential oil.

Plant extraction

The ethanolic, aqueous and ethyl acetate extracts of the plant were provided by soxhlet methods for 4h. and concentrated under vacuum to drying. The extracts and essential oil were stored at -20°C until experiment.

Gas Chromatography (GC) of essential oil

The analysis of the essential oil was performed on an gas chromatograph Agilent HP-6890 (Agilent Technologies, Palo Alto, CA, USA) with a HP-1MS 5% phenylmethylsiloxane capillary column (30 m × 0.25 mm, 0.25 µm film thickness; Restek, Bellefonte, PA) equipped with an Agilent HP-5973 mass selective detector in the electron impact mode (Ionization energy: 70 eV). The temperature of injector and detector was set at 250 °C and 230 °C respectively. the temperature of oven was set at 40°C at first for 1 min, and raised to 250 °C at 3°C/min. Hellium was used as carrier (flow rate1ml/min. Diluted samples (1/100 in acetone, v / v) of 1,0 µl were injected manually in the splitless mode.

Gas chromatography/mass spectrometry (GC/MS)

Essential oils analysis was done with the same GC conditions (oven temperature, column, flow rate) using an Agilent-HP 6890 gas chromatograph equipped with an Agilent 5973 mass selective detector in the electron impact mode (70 ev). Injector and MS transfer line temperatures were set at 250 °C and 230 °C, respectively.

Qualitative and quantitative analysis

For identifying the composition of the oil, relative retention time and mass spectra of each component was compared to standards. confirmation of compounds was performed by comparison of their mass spectra and retention indices relative to n-alkanes of C_8-C_{32} with data in the NISt and Wiley library[10].

DPPH free radical scavenging assay

Antioxidant potential of the essential oil and extracts of the plant was evaluated using DPPH scavenging assay. 50µl of different concentrations of each sample was added to 5 ml of methanolic solution of 0.004% DPPH and incubated at room temperature for 30min., absorbance was read at 517nm[11]. Butylated hydroxytoluene (BHT) was used as reference drug. The percent of inhibition was calculated as follow:

$$I\% = (A_{con}-A_{sam})/A_{sam} \times 100,$$

where A_{con} and A_{sam} are the absorbance of control and sample respectively.

Antibacterial effect using disk diffusion method

Antibacterial effect of the essential oil and extracts was studied by disk diffusion method against seven Gram-positive and gram-negative strains of *Staphyloccocus aureus, Ataphyococcus epidermidis, Pseudomons aeroginosa, Proteus mirabilis, Entrobacter aerogenes, Acinetobacter boumannii* and *Citrobacter freundii.* The plant extracts were diluted in alcoholic solvents and prepared in different concentration, of which 20 µl was dropped on disk of 6 mm in diameter and to give 100µg/disk and 10µl/disk of plant extracts and essential oil respectively.

Turbidities of tested bacterial strains were prepared to 0.5 McFarland and inocula were spread on Mueller Hinton Agar (MHA) and incubated at 37 ºC for 18-24 hours[12]. Inhibition zone were measured using vernier caliper. The tests were undertaken triple and the results were reported as mean ± SD.

RESULTS AND DISCUSSION

GC/MS analysis of essential oil

The essential oil obtained from *D. revoluta* was pale yellow in with distinct, sharp odor. Forthy –eight compounds were identified, representing 94.51% of the total oil components (Table 1). As shown, the major components of the oil were found to be acetophenone (29.94%0), acetylphenol (20.23%), benzaldehyde (14.26%). Phenyl propanoids and benzene derivatives comprise the highest percentage of the total oil (66.21%). Oxygenated sesquiterpenes constitute about 12.00% of the oil. Our results showed differences in composition with respect to data in the literature, such as those reported for the essential oil derived from the *D. diapensifolia.* It is for the first time that the composition of the essential oil of *D. revoluta* has been reported.

Antioxidant activity

The results of antioxidant effect show that ethanolic extract and essential oil of *D. rivoluta* can potentially reduce the stable DPPH radical to yellow color. The highest potential of DPPH radical scavenging was due to the ethanolic extract with IC_{50} value of 98.8 µg/ml in comparison to BHT (IC_{50}= 47.19). The essential oil, aqueous and ethyl acetate extract exhibited inhibition with IC_{50} values of 0.77µl/ml, 126.0 and 264.8µg/ml respectively in a dose dependent manner. The highest percentage of DPPH inhibition of ethanolic extract was 81.6% (200 µg/ml).The lower antioxidant activity of fractions like essential oil or ethyl acetae extract might be due to the absence and/or lower amount of the donor groups such as phenolics or terpenes in these fractions. Monoterpenes with methylene group exhibit potent antioxidant effect which attributed to the presence of methylene groups in monoterpene hydrocarbons[13].

Results of antibacterial effect

The result of antibacterial activity of the essential oil and different extracts of *D. rivoluta* have been shown in Table 2. The ethanol extract showed antibacterial activity against several of Gram-positive and Gram-negative strains except *E.aerogenes* and *C.freundii.* Amongst the tested strains, *A. boumannii* and *S. aureus* showed most sensitivity to ethanolic extract (inhibition zone diameter 11 and 12 respectively). The ethyl acetae extract exhibited weaker effect than that provided by ethanolic extract against *S. aureus, A. baumannii, P. earoginosa* and *P. mirabilis.* This fraction and the essential oil have more prominent inhibitory effect against *C. freundii.* and *A. boumannii* The other strains showed resistance to the essential oil. All the other strains were resistant to essential oil except *E. aerogenes* and *C.freundii* bacteria. No antibacterial effect was shown by aqueous extract.

CONCLUSION

It is the first report for the antioxidant and antibacterial effects of the genus *Dionysia*, and it is the second study for the

chemical composition of this genus. Our results indicated that the ethanolic extract of this plant would be a good candidate for further studies. The essential oil of this plant also exhibited notable antioxidant which needs to be studied using the other methods to have valid results.

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