

Full Length Research Paper

Relationship between antioxidant properties and phenolics in *Zhumeria majdae*

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Zhumeria majdae Rech.f and Wendelbo is a unique plant which is only distributed in south of Iran (Hormozgan province). In present study, the antioxidant activities and phenolic compounds of *Z. majdae* fractions and subfractions have been investigated. The plant material was extracted by ethanol. The fractionation was carried out by using liquid-liquid extraction. Then the ethyl acetate fraction was submitted to further fractionation by using MPLC. Five subfractions (1 - 5) were collected. The reducing power of the ethyl acetate fraction was more than all other subfractions. Ethyl acetate subfraction 2 possessed the greatest radical scavenging activity ($IC_{50} = 41.85 \pm 0.61 \mu\text{g/ml}$) among all of the *Z. majdae* subfractions. No significant differences exist between the IC_{50} of this subfraction and quercetin ($IC_{50} = 38.84 \pm 0.84 \mu\text{g/ml}$), $P > 0.05$. The greatest amount of phenolic compounds ($1.98 \pm 0.01 \text{ mg/g}$) and flavonoids ($357.4 \pm 18.7 \mu\text{g/ml}$) were detected in ethyl acetate subfraction 2.

Key words: *Zhumeria majdae*, antioxidant activity, reducing power, free radical scavenging activity, phenolic compounds.

INTRODUCTION

Antioxidants can minimize or prevent lipid oxidation in food products. Synthetic antioxidants such as tert-butylhydroxy toluene, tert-butylhydroxy anisole and tert-butyl hydroquinone (TbHQ) have been used to retard lipid oxidation in food. However, such synthetic antioxidants are not preferred due to toxicological concerns (Taha et al., 2004).

Phenolics are ubiquitous secondary metabolites in plants. They comprise a large group of biologically active ingredients (above 8000 compounds) from simple phenol molecules to polymeric structures with molecular mass

above 3000 Da (Marinova et al., 2005). Phenolics possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic as well as ability to modify the gene expression (Marinova et al., 2005). Numerous epidemiological studies confirm significant relationship between the high dietary intake of flavonoids and the reduction of cardiovascular and carcinogenic risk (Marinova et al., 2005).

The plant kingdom consists of a wide range of natural antioxidants. However, there is not enough knowledge about medical usefulness of them (Ljubuncic et al., 2006). Therefore, it is of interest to investigate the antioxidant properties of herbal infusions especially those traditionally used in folk medicine (Katalinic et al., 2006) such as *Zhumeria majdae*. Thus, in this research the antioxidant activities of *Z. majdae* (ZM) subfractions were investigated.

Z. majdae is one of the members of Labiatae family which has a limited geographic range in southern region of Iran (near Persian Gulf). This plant is known as Mohr-khosh in Hormozgan province and is used for the

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Abbreviations: DPPH, 2, 2-diphenyl-1-picrylhydrazyl radical, IC_{50} , Inhibition Concentration by 50%; TbHQ, Tert-butyl hydroquinone; ZM, *Zhumeria majdae*; MPLC, Medium Pressure Liquid Chromatography; TCA, Tri Chloro Acetic Acid.

treatment of a wide range of disorders such as diarrhoea, cold, reflux and headache. It is also used as carminative and for wound healing (Izaddoost et al., 1983).

The leaves of this plant are used as an herbal tea, antiseptic and analgesic agent (Izaddoost et al., 1983). The constituent of ZM leaves contain two flavones and 6-methoxy apigenin (Izaddoost et al., 1983) which possessed antioxidant activities (Saskia et al., 1996).

EXPERIMENTAL

Materials

DPPH (2,2-diphenyl-1-picrylhydrazyl radical), quercetin, gallic acid and Folin-ciocalteu reagent were obtained from Sigma Chemical Co., St Louis, MO. All other reagents were obtained from Merck Chem.

Plant extraction and fractionation

The leaves of ZM were purchased from local medicinal plants store in Bandar-e-Abbas. The plant materials were identified and authenticated by Dr. Moein in the Department of Pharmacognosy at Shiraz University of Medical Sciences, Shiraz, Iran. *Z. majdae* aerial parts (1.9 kg) were extracted with n-hexane and methanol (20 lit), respectively. Defatted methanol extract of ZM was dissolved in methanol (80%) and fractionated with chloroform, the residue was concentrated and dissolved in water and extracted with ethyl acetate and n-butanol, respectively. 4.9 g of ethyl acetate fraction was subjected to further fractionation by using MPLC (Medium Pressure Liquid Chromatography) and eluted by solvent mixture (chloroform: methanol: H₂O 7: 1: 0.01). Five subfractions (1 - 5) were collected based on TLC detection.

Measurement of reducing power

The reducing power of ZM ethyl acetate, fraction and subfractions were determined using the method described by Yen and Duh (1990). Extract of various concentrations was made (12.5 - 200 µg/ml) in 0.2 M phosphate buffer pH, 6.6 containing 1% ferrocyanate. The mixture was incubated at 50°C for 20 min. To 5 ml of this mixture 2.5 ml of 10% TCA (Tri Chloro Acetic acid) was added and centrifuged at 3000 g for 10 min. The supernatant was separated and mixed with 2.5 ml of distilled water containing 0.5 ml of ferric chloride 1%. The absorbance of this mixture was measured at 700 nm. The intensity in absorbance could be the measurement of antioxidant activity of the extract (Yen and Duh, 1990).

Determination of antioxidant using DPPH

The antioxidant activity of ZM (sub) fractions and the antioxidant standard were assessed on the basis of radical scavenging effect of the stable DPPH free radical. Two hundred µl of a 100 mM solution of DPPH radical in methanol was mixed with 20 µl of plant fraction (or subfractions). The concentrations of fractions were 12.5 - 400 µg/ml. After mixing, they were left for 30 min at room temperature.

The DPPH radical inhibition was measured at 490 nm (Moein et al., 2007) by using a micro-plate reader model Stat Fax 2100, Awareness technology, Inc.

The IC₅₀ of each sample (concentration in µg/ml) required to inhibit DPPH radical concentration by 50% is also calculated. Tests were carried out in triplicate.

The antioxidant activity (AOA) was given by:

$$100 - \frac{(A) \text{ sample} - (A) \text{ blank}}{(A) \text{ control}} \times 100 / (A)$$
 where "A" is the absorbance of the color formed in microplate wells. DPPH used as control (without plant fraction), blank contains methanol (Moein et al., 2007).

Determination of total phenolic content

The content of total phenolic compounds in ZM fraction and subfractions were determined by using Folin-Ciocalteu reagent. For the preparation of calibration curve 1 ml aliquots of 0.024, 0.075, 0.105 and 0.3 mg/ml methanolic gallic acid solutions were mixed with 5 ml Folin-Ciocalteu reagent (diluted ten-fold) and 4 ml (75 g/l) sodium carbonate. The absorption was read after 30 min in 20°C at 765 nm and the calibration curve was drawn. One ml of methanol plant extract (10 g/l) was mixed with the same reagents as described above and after 1 h the absorption was measured for the determination of plant phenolics. All determinations were performed in triplicate. Total content of phenolic compounds in plant methanol extracts in gallic acid equivalents (GAE) were calculated (Miliauskas et al., 2004) by the following formula:

$$C = c \cdot V / m \quad (2)$$

where C is the total content of phenolic compounds, mg/g plant extract, in GAE; c is the concentration of gallic acid established from the calibration curve, mg/ml; V is the volume of extract, ml; m is the weight of pure plant methanol extract, g (Miliauskas et al., 2004).

Determination of total flavonoid content

The total flavonoid content of ZM subfractions was determined by using colorimetric method. A 0.5 ml aliquot of appropriately diluted sample solution was mixed with 2 ml of distilled water and subsequently with NaNO₂ 0.15 % solution. After 6 min, 2 ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5 ml, then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm versus a prepared water blank (Yanping et al., 2004). Quercetin was used as a standard compound for the quantification of total flavonoid. All values were expressed as mg of quercetin equiv per 1 g of extract. Data was recorded as mean ± SD for three replications.

Statistical analysis

IC₅₀ values were calculated by linear regression. Means ± SD were calculated. The data were analyzed for statistical significance using one way ANOVA followed by Tukey post test. P values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Z. majdae is a unique plant that belongs to Labiatae family. Analysis of essential oil showed that major constituents are camphor and linalool (Majrouhi, 2009). Also, antileishmanial, antiplasmodial, cytotoxic (Moein et al., 2008 b), antimicrobial on a range of microorganisms including gram negative, gram positive, yeast and fungi (Mahboubi and Kazempour, 2009), antinociceptive, anti-

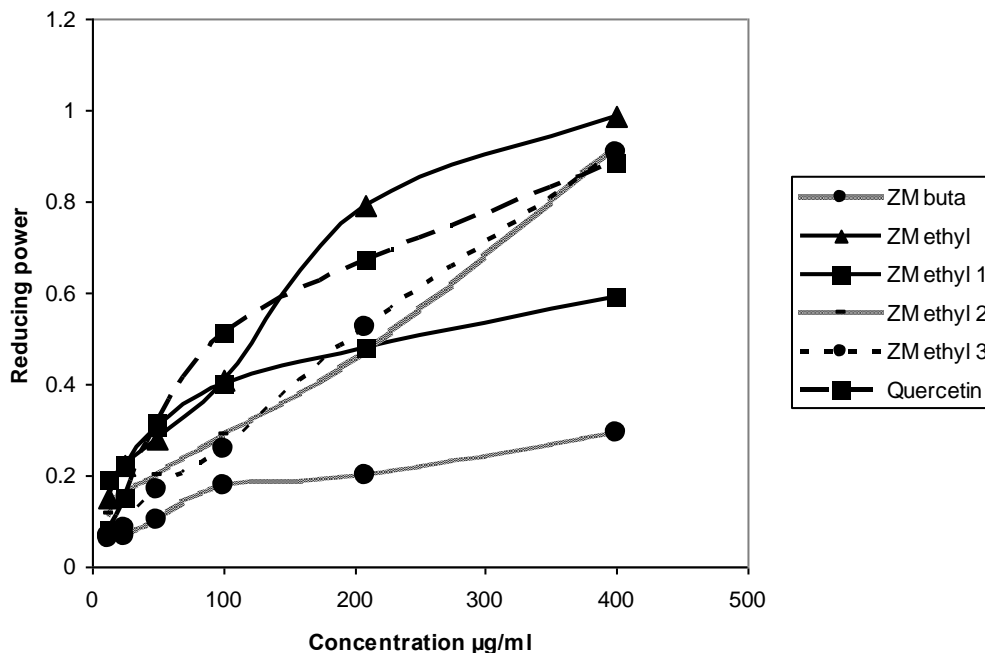


Figure 1. Reducing power of *Z. majdae* subfractions were compared with quercetin as an antioxidant standard which is diluted 1: 10.

inflammatory and acute toxicity effects have been reported (Hosseinzadeh et al., 2002).

Two new diterpene quinones with rearranged abietane skeletons, 12, 16-dideoxy aegyptinone B and 12-deoxy-salvipisone, with a known manool have been isolated from underground part of this plant (Rustaiyan et al., 1995). 12, 16-dideoxy aegyptinone B indicated very strong antileishmanial activity in comparison with pentamidine (Moein et al., 2008 b).

In this research, the antioxidant properties and phenolic compounds of butanol fraction and ethyl acetate (sub) fractions of ZM were investigated. For determination of antioxidant properties, the reducing power and radical scavenging were determined.

Antioxidant compounds can convert the oxidation form of iron (Fe^{+3}) to ferrous (Fe^{+2}) in ferric chloride. This conversion can be detected by reducing power assay (Moein et al., 2008 a).

For precision comparison between ZM subfractions, reducing power is calculated as concentration which produces 0.5 absorbance in the assay. In this research, the reducing power of all ZM subfractions were less than quercetin ($P < 0.001$, Table 1) and this power of ZM ethyl acetate fraction was more than the other subfractions ($P < 0.001$, Table 1 and Figure 1).

In radical scavenging assay, the exposure of DPPH and antioxidant compounds causes the purple colour of DPPH changes to yellow. More yellowish colour of DPPH shows more antioxidant activity of the compounds which was tested (Moein et al., 2008 a). The results of radical scavenging activity showed that, ethyl acetate subfraction

2 possessed the greatest radical scavenging activity among the entire ZM subfraction (Figure 2). Also, the IC_{50} of this subfraction is similar to quercetin ($P > 0.05$, Table 1). In other words, this subfraction in radical scavenging activity is strong as far as quercetin.

The amount of phenolic compounds of ZM subfractions is determined by using the Folin-Ciocalteu reagent. The content of total flavonoids is also measured by using the aluminum chloride colorimetric assay (Moein et al., 2008a). In this research, a correlation between radical scavenging activity and phenolic compounds has not been found ($R = -0.1930$). This result was also found by other researchers (Slusarczyk et al., 2008).

The differences between correlation of polyphenols and antioxidant capacity may be explained by the presence of some chemical groups such as amino acids and proteins that can also react with Folin-Ciocalteu reagent (Meda et al., 2008).

In this research, there was not found any correlation between radical scavenging activity and the amount of flavonoids ($R = -0.1930$). But a significant correlation has been found between total phenolic compounds ($R = 0.9384$) and the amount of flavonoids. This result may be showed that the phenolics types of ZM subfractions which possess antioxidant activity are flavonoids. On the other hand in other researches, a low coefficient correlation ($R = 0.048$) between phenolic compounds and flavonoids was reported (Meda et al., 2008; Moein et al., 2008a).

In present research, the highest amount of phenolic compounds was in ethyl acetate subfraction 2 ($1.98 \pm$

Table 1. Reducing power, radical scavenging, phenolic and flavonoids content of *Zhumeria majdae* subfractions.

Samples	Reducing power ^a	Radical scavenging IC ₅₀ µg/ml ^b	Total phenolic content mg/g ^c	Total flavonoid content µg/ml ^d
Crude extract	ND	NA	0.828 ± 0.075	3.8 ± 0.03
ZM. ethyl acetate fraction	122.2 ± 0.006	59 ± 1.18	0.98 ± 0.095	155.2 ± 5.35
ZM. ethyl acetate subfraction 1	339 ± 0.006	NA	1.51 ± 0.01	NA
ZM. ethyl acetate subfraction 2	211.9 ± 0.08	41.85 ± 0.61	1.98 ± 0.01	357.4 ± 18.7
ZM. ethyl acetate subfraction 3	>400	138.34 ± 10.04	1.32 ± 0.005	115.56 ± 3.47
ZM. ethyl acetate subfraction 4	ND	NA	ND	ND
ZM. ethyl acetate subfraction 5	ND	ND	ND	ND
ZM. butanol fraction	>400	47.9 ± 3.03	ND	1.2 ± 0.006
Quercetin	10 ± 0.06	38.84 ± 0.86	ND	ND

^a Concentrations of *Z. majdae* subfractions in absorbance 0.5 compared with quercetin as standard in reducing power assay, ^b IC₅₀ of *Z. majdae* fractions compared with standard of quercetin, ^c the amount of total phenolics in *Z. majdae* subfractions and ^d the amount of total flavonoids in *Z. majdae* subfractions. Key: ND = not determined; NA = not Active.

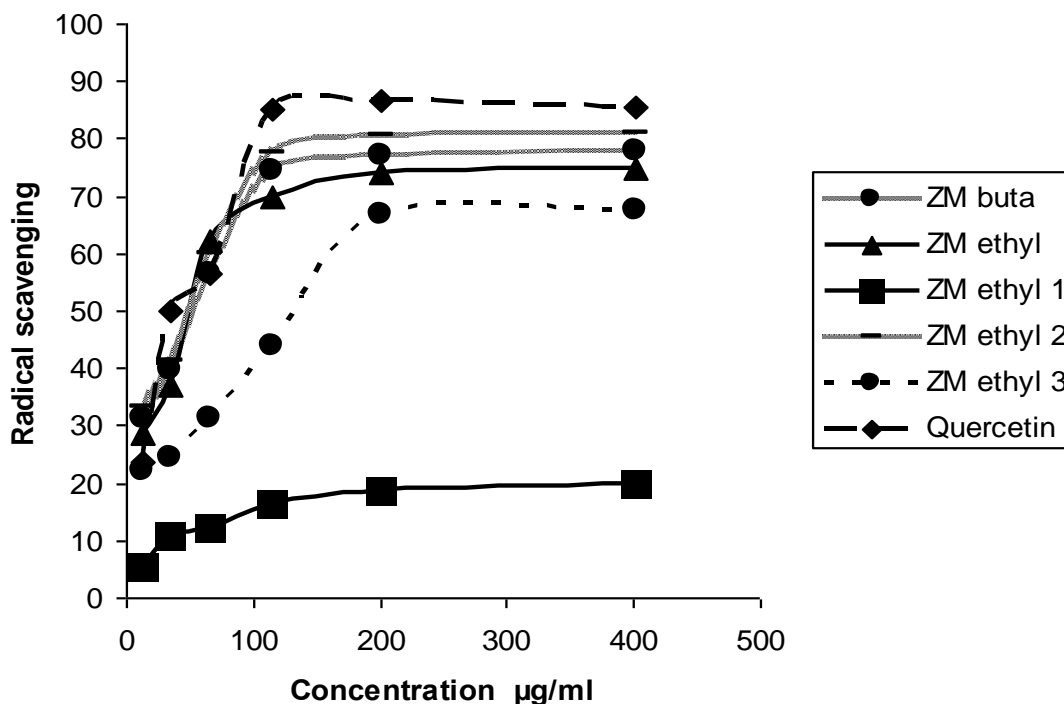


Figure 2. Radical scavenging activity of ethyl acetate fraction of *Z. majdae* and its fractions compared with quercetin as a standard.

0.01 mg/g, Table 1) and the lowest amount of phenolics was in ethyl acetate fraction (0.98 ± 0.095 mg/g, Table 1).

The highest amount of flavonoids was in ethyl acetate subfraction 2, (357.4 ± 18.7 µg/ml) and the lowest amount of flavonoids was in butanol fraction (1.2 ± 0.006 µg/ml).

Any way, the correlation between antioxidant activities of plant extracts and their individual phenolic compounds can be used as a clue for prevention of oxidation (Taha et

al., 2004).

Conclusion

By using MPLC, five subfractions of ZM ethyl acetate were eluted. Radical scavenging activity of one of these subfractions, subfraction 2 (IC₅₀ = 41.85 ± 0.61 µg/ml) is similar to quercetin (IC₅₀ = 38.84 ± 0.86 µg/ml). Also this

subfraction possesses the greatest amount of phenolic (1.98 ± 0.01 mg/g) and flavonoid (357.4 ± 18.7 μ g/ml) compounds.

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