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In Vitro Antioxidant Activity and Total Phenolic and Flavonoid Contents of Alhydwan (*Boerhavia elegana Choisy*) Seeds

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Abstract Alhydwan seeds are widely used by indigenous tribes in the traditional cuisines of South Yemen. The objectives of this study were to determine antioxidant components and antioxidant activities of ethanolic extract of Alhydwan (*Boerhavia elegana Choisy*) seeds cultivated in Yemen for human consumption (novel plant). The work was done by using several in vitro methods such as 1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2, 2-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), β-carotene, reducing power and chelating activity. Were also determined total phenolic content by the Folin-Ciocalteu colorimetric and Flavonoid content using catechol. Results showed that *B. elegana Choisy* seeds had the highest phenol (253.9 ± 0.9mg/1 g), flavonoids (23.68 ± 0.6 mg/1 g), contents; total phenolic content was the most abundant in seeds. *B. elegana Choisy* seeds exhibited potent DPPH, ABTS, β-carotene bleaching and metal chelating except reducing power. Effect was also increasing with an increase in *B. elegana Choisy* concentration. The findings of this study have clearly demonstrated Alhydwan (*Boerhavia elegana Choisy*) seeds possess high phenolic, flavonoid contents and potential antioxidant activity, and could be used as a viable source of natural antioxidants and might be exploited for functional foods.

Keywords: *Boerhavia elegana Choisy, antioxidant activity, total phenolic content, total flavonoid compounds, novel plant*

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1. Introduction

Antioxidants and pro-oxidants are compounds that can accelerate or delay oxidation processes. Nowadays the interest in natural antioxidants has increased considerably because of their beneficial effects of prevention and risk reduction in several diseases [1]. Recently, numerous studies have described antioxidants and compounds with radical scavenging activity to be present in fruits, seeds, vegetables, herbs and cereals extracts [2,3,4]. Antioxidants of natural origin have attracted increasing interest due to they can protect human body from free radicals without producing toxic effects [5]. It is well known that consumption of dietary antioxidants is associated with reduced risk of several diseases in which oxidative stress may play a role, especially chronic diseases such as cardiovascular, cancer and neurodegenerative and inflammatory diseases [6]. Currently, it is an increasing interest in finding natural antioxidants from plant materials to replace synthetic ones [7]. This is due to the fact that natural antioxidant substances are safe since they occur in plant foods and are more desirable than their synthetic counterparts, thus, there is a considerable interest in finding natural antioxidants from plants [8]. Hence, the research on antioxidants has become an important scientific topic in food, pharmaceutical and cosmetic fields [9]. Studies have shown that plants contain a variety of substances, which possess antioxidant activity, such as vitamin C, phenolics, carotenoids and tannins [8]. Phenolic compounds are considered from natural antioxidants, and are biologically active substances [10]. Common bean (*Phaseolus vulgaris* L.) is a traditional food in human diet, rich in proteins, minerals, complex carbohydrates and vitamins, and low in fat. It is well known that the consumption of the common bean is one of the most widespread practices around the world, accounting for almost half of the consumed legume grains, especially in the less developed countries [11]. Also it's grown and consumed in various regions of the world, It is becoming increasingly popular because of its health benefits, and providing an inexpensive source of protein in the diet [12].

Boerhavia elegana Choisy seeds (common name: alhydwan) is an edible herbaceous member of the Nyctaginaceae family and is commonly available in South Yemen [13]. It is widely used by indigenous tribes in the traditional cuisines of South Yemen, which is one of a staple ingredient in the manufacture of porridge, it is eaten

as supplement in the mixtures of bread and cakes which is characterized by good flavor, Furthermore, and it is one of the ingredients in the manufacture of desserts and savory products. While some species from genus *Boerhavia* have been widely used in the traditional system of medicine in many country [14]. Previous study have reported substantial amounts of total phenolic and flavonoid contents and antioxidant activity on species of *Boerhavia diffusa* from the same genus [15,16]. However, to our knowledge, no research has been done up to now or any information available in the literature on species (*Boerhavia elegana Choisy)* for antioxidant activity and total content of phenolic and flavonoid.

The objectives of this study therefore, were to (i) examine the antioxidant properties of *B. elegana Choisy* seeds from Yemen; (ii) determine the phenolic and flavonoid contents; and (iii) provide evidence for of alhydwan seeds as a good quality novel plant.

2. Materials and methods

2.1. Materials and Reagents

Dried alhydwan seeds were brought from a farmland in Hadramout city, Yemen, in December, 2013, and transferred to the Department of Food Science and Technology, Faculty of Engineering and Technology, Jiangnan University, Wuxi City, People's Republic of China. Methanol, chloroform, Ethanol, sodium nitrite, $AlCl_3$, $FeSO_4$, $FeCl_2$, $FeCl_3$, NaOH, Na_2CO_3, potassium persulphate, sodium carbonate, perchloric acid, and HCl. Were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Chlorogenic acid, b-Carotene, quercetin, ABTS solution, butylatedhydroxytoluene (BHT), linoleic acid, Tween 20, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, acetate buffer, ferrozine, EDTA, gallic acid, and Folin–Ciocalteu reagent, were purchased from Sigma-Aldrich (St. Louis, MO).All chemicals and reagents in the investigation were of analytical grade.

2.2. Samples Preparation and Extraction

The dry alhydwan seeds were milled into powder to pass through 100-mesh sieve, sealed in polyethylene bags and stored in a refrigerator at 4°C until use. To obtain an extract, 50 g of sample was mixed with 150 mL of 95% ethanol in a blender for 1 min and shaken in the dark at 25°C for 4.5 h. The mixture was then centrifuged for 20 min at 4°C and the supernatant filtered. The extract was evaporated to dryness using a rotary evaporator (IKA, RV10-German). The dry extract was drained under a nitrogen stream and was then stored in a refrigerator at 4°C till analysis.

2.3. Determination of Total Phenolic and Flavonoid Contents

Total phenolic content (TPC) was analyzed by the Folin-Ciocalteu colorimetric method using gallic acid as standard and expressed as mg/g gallic equivalent [17]. Flavonoid content was analyzed using catechol as standard and this was expressed as mg/g catechol [18].

2.4. DPPH Radical Scavenging Activity Assay

The DPPH Radical scavenging activity was determined according to the technique reported by [19] with a slight modification. Extract concentrations of 1, 2, 4, 8, and 16 mg extract equivalents/ mL were mixed with 3.5mL of DPPH solution and the absorbance was read at 517nm. The mixtures were incubated for 30 min at 25°C. Then, the absorbance was recorded at 517nm. Ascorbic acid was used as the standard. The DPPH radical scavenging activity was calculated according to the following equation:

$$\text{DPPH Scavenging activity } \% = \left(A_c - A_s / A_c\right) \times 100$$

Where:

A_c is the absorbance of the control and A_s is the absorbance of extract.

2.5. ABTS Radical Scavenging Assay

ABTS assay of *B. elegana Choisy* seeds was based on the method of [20] with slight modifications. Briefly, an aliquot of extract 1, 2, 4, 8 and 16mg extract equivalents/mL concentrations were mixed with 900 µL of 100mM Tris-HCl buffer (pH 7.4), 40 µL of methanol and 50 µL of 0.5% (w/w) Tween 20 solution, an ABTS radical was generated by mixing 7 mM ABTS and 2.45 mM potassium persulphate via incubation at 23°C in the dark for 12 h. Then, 0.1 mL of the sample solution was mixed with 2.6 mL of a diluted ABTS radical solution. The absorbance of the solution was recorded at 734nm after incubation at 23°C for 6 min. ABTS radical scavenging assay was calculated using the formula: scavenging activity % = (Ac–AS / Ac) ×100, where Ac is the absorbance of the control and As is the absorbance of extract. BHT was used as the standard compound.

2.6.β-Carotene Bleaching Test

Antioxidant activity was determined also using the β-carotene bleaching test by the method of [21] an amount of one mL of β-carotene solution in chloroform (3.34 mg/mL) was pipette into a flask containing 50 mg linoleic acid and 500 mg Tween 20. The chloroform was removed by rotary evaporation at 40°C for 5 min and 100 mL of distilled water were slowly added to the solution with vigorous agitation to form an emulsion. A 5 ml aliquot of the emulsion was added to a tube containing 0.2 mL of the antioxidant solution at 200 mg/L and the absorbance was measured at 470 nm, immediately, against a blank consisting of the emulsion without β-carotene. The tubes were then placed in a water bath at 40°C and the absorbance measurements were made again at 30 min intervals. BHT was used for comparison.

2.7. Reducing Power Assay

The reducing power of the extract was based on the method of [22] with slight modification. Extract concentrations of 1, 2, 4, 8 and 16 mg Extract equivalents/mL were mixed with potassium ferricyanide (200 µL, 10 mg/mL^{-1}) and a sodium phosphate buffer (200 µL, 0.2 M, pH 6.6) and incubated at 50°C for 30 min. Trichloroacetic acid (200 µL, 100 mg/mL^{-1}) was added and the mixtures were again incubated for 5 min to stop

the reaction. Then, 680 µL of the reaction mixtures were mixed with 680 µL of distilled water and 68 µL of ferric chloride (10 mg/mL). Absorbance of solution was recorded at 700 nm Ascorbic acid (0.5 mM) was used standard.

2.8. Iron-chelating Ability Assay

The chelating ability of *B. elegana Choisy* seeds extracts for ferrous ions Fe^{2+} was measured according to the method previously described by [23] with some modifications. To 0.5 mL of extract in deionized water at different concentrations (1, 2, 4, 8, and 16 mg/mL), 1.6 mL of deionized water and 0.05 mL of $FeCl_2$ (2 mM) were added. After 30 s, 0.1 mL ferrozine (5 mM) was added. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. After 10 min at room temperature, the absorbance of the Fe^{2+}–Ferrozine complex was measured at 562 nm. The chelating ability of the extract for Fe^{2+} was calculated using the following equation:

$$\text{Chelating rate} = \left(A_c - A_s / A_c\right) \times 100$$

Where:
A_c is the absorbance of the control and
A_s is the absorbance of extract.

3. Results

3.1. Total Phenol and Flavonoid Contents

The results showed that the *B. elegana Choisy* seeds ethanolic extract, possessed high phenolic content when measured by Foline-Ciocalteu reagent in terms of gallic acid equivalent (253.9±0.9 mg/g) and flavonoid content calculated as catechol equivalent (23.68±0.6 mg/g). In the present study, there are good indications that the phenolics of this plant are important components which can have the effect of pharmacological invaluable. It is well known that the flavonoids show antioxidant activity and their effects on human nutrition and health is significant.

3.2. DPPH Free Radical Scavenging Activity

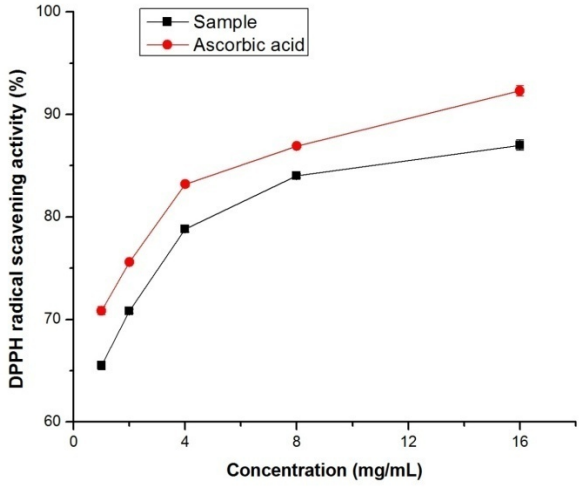

Figure 1. Radical scavenging assay of *B. elegana Choisy* by DPPH. Values are mean ± standard deviation of three determinations

DPPH assay is considered one of the most methods which are used widely for screening antioxidant activity of plant extracts. The results of DPPH radical scavenging activity of *B. elegana Choisy* and the reference antioxidant (Ascorbic acid) are presented in the Figure 1. The seed extract and the reference antioxidant (Ascorbic acid) promoted an inhibition of DPPH radical with increasing concentrations. *B. elegana Choisy* ethanolic seed extract showed a significant effect in inhibiting DPPH, reaching up to 87.00% and 92.3% for the seed extract and standard antioxidant (ascorbic acid), respectively at the highest concentration. IC_{50} of the sample was (2.42) while control was (1.47). This method is based on scavenging of the DPPH radical from the antioxidants, which produces a decrease in absorbance at 517 nm.

3.3. ABTS Radical Scavenging Assay

The antioxidant activity of *B. elegana Choisy* was also studied using the ABTS assay. The results of the ethanolic seed extract showed strength and effectiveness in scavenging the ABTS radical as shown in Figure 2, B. elegana Choisy ethanolic seed extract showed a significant effect in inhibiting ABTS, reaching up to 89.3% and 93.4% for the seed extract and standard antioxidant (BHT). Respectively at the highest concentration, and this activity was close to that of standard BHT. IC_{50} of the sample was (2.24) while standard was (1.86).

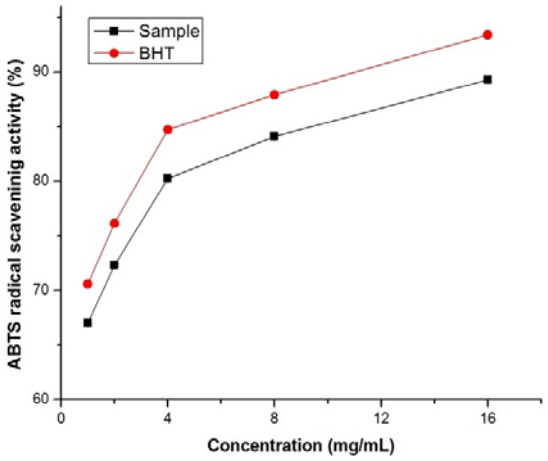

Figure 2. Radical scavenging assay of *B. elegana Choisy* by ABTS. Values are mean ± standard deviation of three determinations

3.4. β-Carotene Bleaching Test

The β-carotene bleaching inhibition effect of the extract of B. elegana Choisy seeds and the standard compound (BHT) are illustrated in Figure 3. The anti-bleaching activity of β-carotene was estimated by surveillance of color intensity of the emulsion recorded at 470 nm at every 30 min for 2 h. The absorbance of both the sample extract and the standard (BHT) at 0 min was 0.245 nm. The concentration of the Extract and the standard (BHT) absorbance of the sample was 0.245 nm. Then, after the next 30 min the sample showed an absorbance of 0.22 nm bleaching as compared to that of the reference (BHT) at 0.26. After 90 min of incubation, the absorbance decreased to 0.183 nm and 0.23 nm for the sample and the reference (BHT), respectively. Eventually, at the last 120 min, the absorbance was 0.136 and 0.21 nm for the sample and standard (BHT), respectively.

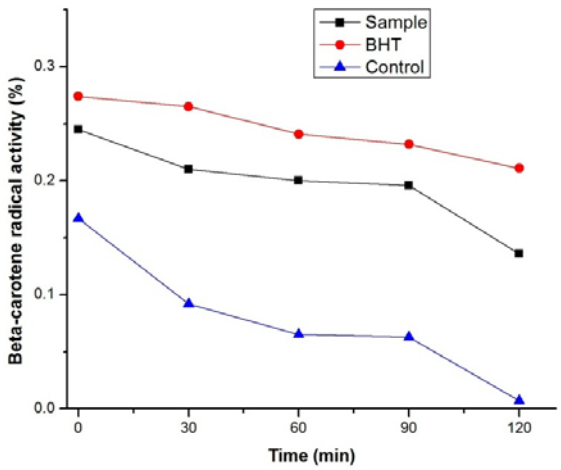

Figure 3. β-Carotene bleaching activity of *B. elegana Choisy.* Values are mean ± standard deviation of three determinations

3.5. Reducing Power

The reducing power results of ethanolic extract of *B. elegana Choisy* and reference antioxidant (BHT) are presented in the Figure 4. Both the seeds extract and BHT showed increased absorbance with of increasing concentration.

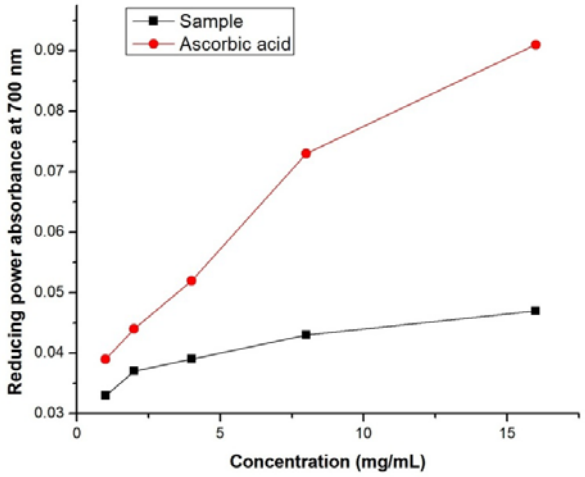

Figure 4. Reducing power of *B. elegana Choisy.* Values are mean ± standard deviation of three determinations

3.6. Iron-chelating Ability

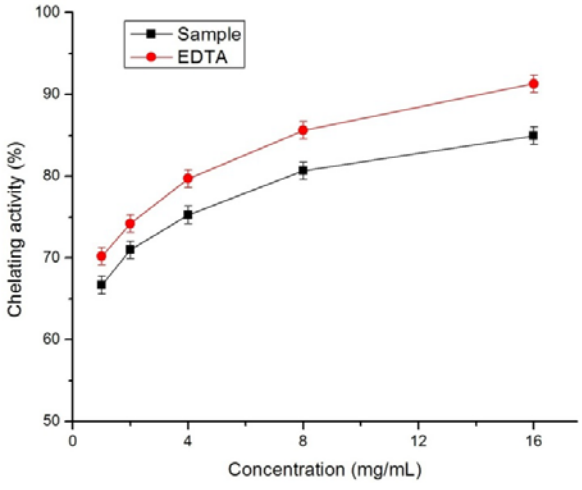

Figure 5. Iron chelating ability of *B. elegana Choisy.* Values are mean ± standard deviation of three determinations

Figure 5 presents the iron chelating ability of both *B. elegana Choisy* seeds and reference antioxidant (EDTA), the data obtained of *B. elegana Choisy* extract exhibited potent and increases as the concentration increases, The minimum in vitro chelating ability of the seeds extract was 66.67% At the lowest concentration and the maximum capacity 84.95% at the highest concentration, and was seeds extract chelated less iron than efficient than commercial chelator. IC_{50} of the sample was (6.78) while control was (6.35).

4. Discussion

4.1. Total Phenol and Flavonoid Contents

Plants are rich sources of natural antioxidants such as phenolics and tocopherols, which are considered to be the best-known components [24]. Phenolic compounds are highly reactive toward free radicals, Hence, quantification of these compounds is also indicative of antioxidant activity [25].It is known that Phenolic compounds from plants are good natural antioxidants. The high phenolic compounds value obtained in *B. elegana Choisy* seeds is in agreement with the study that analyzed various species of genus *Boerhaavia diffusa* root [16]. also the value obtained for total phenol content, from this study is higher than the values reported Rocha-Guzmán et al [39] studied total phenol content in three different of common bean. Polyphenolic compounds are antioxidants and may help to prevent diseases associated with oxidative stress, such as atherosclerosis, cancer and neurodegenerative diseases [26,27,28]. The value obtained for total flavonoid content from this study was high and this is considered an advantage because of the plants containing phenolic compounds, especially the flavonoids have exhibit strong antioxidant properties [29]. It is also reported by [30,31] that the radical scavenging activity is an indicator of the functionality and antioxidant activity and can be attributed to the contents of total polyphenols and total flavonoids in plant foods.

4.2. DPPH Free Radical Scavenging Activity

From Figure 1, *B. elegana Choisy* demonstrated the capacity for scavenging free radicals by reducing the stable DPPH radical to the yellow colored diphenylpicryl hydrazine. These findings indicated the potential electron and/or hydrogen donating ability of *B. elegana Choisy* extract. Based on the scavenging capacity of the free radicals, the highest antioxidant activity was found in *B. elegana Choisy extract.* Value of DPPH scavenging activity which found from this study is higher than the values those found by Zhao et al [42] studied the DPPH scavenging activity in 10 kinds of beans. This may be attributed to the presence flavonoids that exhibit strong antioxidant properties [29]. Moreover, [32] reported that antioxidant activity of plant extracts containing polyphenolic compounds have capacity to be donate hydrogen atoms or electrons and to capture the free radicals. However, the radical scavenging potential of the reference antioxidant (Ascorbic acid) was higher than that of the extract.

4.3. ABTS Radical Scavenging Assay

The ABTS method depends on the inhibition of the absorbance of radical cation ABTS, which has a feature wavelength at 734 nm. Decolonization of ABTS reflects the capacity of the antioxidant species to donate electrons or hydrogen atoms to inactivate these radical actions. In the presence of antioxidant reductant, the colored radical is converted back to colorless ABTS [33]. The result obtained in this study in ABTS is higher than the values reported by Marathe et al [41] in Common Beans. Also the result of ABTS radical scavenging activities of the extract was comparable to those observed from DPPH assay. ABTS radical scavenging activity is relatively recent, often used for screening complex antioxidant mixtures such as plant extracts, and involves a more drastic radical, chemically produced [15].

4.4. β-Carotene Bleaching Test

In this assay of β-Carotene bleaching it lost yellow of β-carotene because of the interaction between β-carotene with radicals created by linoleic acid oxidation in an emulsion [34]. The result obtained in this study in a β-carotene is higher than the values reported by Cardador-Martínez, et al [40] in some common beans. In the β-carotene/linoleic acid test has been oxidized the highly unsaturated fatty acids due to the oxidation of linoleic acid which generates peroxyl free radicals. This is an indicator of the presence of antioxidants which minimize the oxidation of β-carotene compounds.

4.5. Reducing Power

In this method the reducing potential of *B. elegana Choisy* extract was evaluated. The reducing power reflects the electron donating capacity of its bioactive compounds, which serves as a significant indicator of its antioxidant activity. The results exhibited that *B. elegana Choisy* extract, lesser than standard antioxidant, value of reducing power of alhydwan seeds are similar to values of some common legumes, reported by Zhao et al [42] studied the reducing power of 10 kinds of beans. Reduced Fe3+/ferricyanide complex to the ferrous form, which indicated existence of reductants in the sample solution. The presence of these reductants in *B. elegana Choisy* was considered keys to its reducing power, because they appear as antioxidants by donating a hydrogen atom and breaking the free radical chain [35].

4.6. Iron-chelating Ability

The iron-chelating ability assay is considered of importance to screen the iron and mechanism of antioxidant activity and its ability to chelate/deactivate transition metals, it possesses ability to catalyze hydrogen peroxide decomposition and Fenton-type reactions [36]. The value of metal chelating which found in alhydwan seed are close to those found by Carrasco-Castilla et al [12] in from phaseolin and bean protein which were (78% and 82%).

It could be attributed the antioxidant activity to the presence of bioactive constituents [37]. Such as total polyphenols, total flavonoids, radical scavenging activity, antioxidant capacity and Fe-chelating activity [25]. Furthermore, phenolic compounds are responsible of Fe chelating activity and a high correlation exists between Fe-chelating ability with flavonoids and phenolic compounds exhibited redox properties (i.e. serves as reducing agents, hydrogen donators and singlet oxygen quenchers) [38,30,31].

5. Conclusion

The findings of this study have clearly demonstrated the interesting antioxidant properties of *Boerhavia elegana Choisy* seeds. The seeds extract exhibited potent the most promising free radical scavenging effects evaluated by DPPH and ABTS tests and a good antioxidant activity through different mechanisms of action (FRAP assay, b-carotene bleaching test, Fe^{2+} chelating assay). The seeds extract was characterized by the high content of phenols and flavonoids signifying potent antioxidant properties. Eventually, the results showed that *Boerhavia elegana Choisy* seeds may be used as a source of healthy compounds for the development of dietary supplements and to protect against oxidative stress besides used for food consumption.

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