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Analysis of Al Haydawan (*Boerhavia elegana)* Seed Extracts: Mineral Composition, Total Phenolic Content, and Primary Antioxidant Activity

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Abstract: Many consumers are increasingly seeking safer alternatives to synthetic additives in food, leading to a preference for healthier options. Consequently, the food industry is exploring various plant-based preservatives. These natural ingredients offer antioxidant benefits, boost bioactive compound levels, and enhance food products' microbiological stability. It is also crucial to assess how these preservatives influence the sensory attributes of new items, as they may produce flavors or textures that some consumers find unappealing. *Boerhavia elegana* L. (from the Nyctaginaceae family) is a medicinal plant known for treating kidney issues, urinary problems, and blood purifying. This study investigated the differences between aqueous and ethanolic AlHaydawan extracts regarding mineral, antioxidant, and phenolic content on cold storage's first and seventh days.

Keywords: Al Haydawan (*Boerhavia elegana)*, Minerals, Total phenolic content, antioxidants, solvent extraction.

تحليل مستخلصات بذور الهايداوان (Boerhavia elegana): التركيب المعدني، المحتوى الفينولي الكلي، والنشاط المضاد للأكسدة الأساسي

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المستخلص: يبحث العديد من المستهلكين بشكل متزايد عن بدائل أكثر أمانًا للمضافات الصناعية في الأغذية، مما يؤدي إلى تفضيل الخيارات الصحية. ونتيجة لذلك، تستكشف صناعة المواد الغذائية العديد من المواد الحافظة النباتية حيث توفر هذه المضافات الطبيعية فوائد مضادة للأكسدة، وتعزز مستويات المركبات النشطة بيولوجيًا، وتعزز الاستقرار الميكروبيولوجي للمنتجات الغذائية. ومن المهم أيضًا تقييم كيفية تأثير هذه المواد الحافظة على السمات الحسية للعناصر الجديدة، لأنها قد تنتج نكهات أو قوام يجده بعض المستهلكين غير جذاب (*Boerhavia elegana*) من عائلة (Nyctaginaceae) هو نبات طبي معروف بعلاج مشاكل الكلى ومشاكل المسالك البولية وتنقية الدم. تناولت هذه الدراسة الفروق بين مستخلصات الحيدوان المائية والإيثانولية فيما يتعلق بالمحتوى المعدني ومضادات الأكسدة والفينولات في اليومين الأول والسابع للتخزين البارد.

الكلمات المفتاحية: الهايداوان (Boerhavia elegana)، المعادن، المحتوى الفينولي الكلي، مضادات الأكسدة، الاستخلاص بالمذيبات

1- Introduction

In today's health-conscious society, people are becoming increasingly mindful of their well-being and the nutritional content of their foods. Among the various nutrients, antioxidants have a significant popularity because of their ability to combat and prevent various physiological diseases. Antioxidants are effectively slow down or prevent the oxidation of an oxidizable substance when found in lower amounts relative to the substrate.(Li *et al.*, 2007).

Antioxidants from plants, particularly phenolics, are becoming increasingly important due to their potential health benefits. Research has indicated that eating plant-based foods rich in antioxidants can positively impact health. This consumption helps reduce many degenerative conditions and can significantly decrease the risk of cancer and heart diseases (Arabshahi and Urooi, 2007). Solvent extraction is the predominant method for isolating antioxidant compounds from plants. The antioxidant activity of these extracts are heavily influenced by the choice of solvent. Different antioxidant compounds possess varying chemical properties and polarities, affecting their solubility. Methanol and ethanol are commonly utilized for the extraction of antioxidant compounds from different plants and plant-based foods, including fruits and vegetables like plums, kernels, and citrus peels. Additionally, other research has shown that ethyl acetate can effectively extract phenolic compounds from onion and citrus peels (Rehman, 2005).

Bonoli *et al.*, (2004) found that combining ethanol and acetone effectively extracted most phenolic compounds from barley flour. Similarly, Chatha *et al.* (2006) found that a mixture of methanol and water was particularly effective in extracting the highest phenolic compounds from rice bran, while Siddhuraju and Becker (2003) reported similar results for Moringa leaves. Anwar *et al.*, (2006) used 80% methanol in water (80:20 v/v) to extract antioxidant compounds from a range of plants, including rice bran, wheat bran, oatmeal and husks, coffee beans and citrus.

Boerhavia elegana is a perennial plant that grows as an upright, smooth shrub reaching up to 1 meter in height, supported by a strong root system. Its thick stems become woody near the bottom, displaying a dark green color, often tinged with red. The stems are mostly hairless or have a few hairs and branch out mainly from the base, with the joints being swollen. Antioxidants are important substances that can slow the oxidation process by preventing or interrupting the chain reaction caused by free radicals. Natural antioxidants offer a great option for food additives. In addition to effectively eliminating free radicals, their natural origin makes them a healthier and safer choice compared to synthetic alternatives. As a result, they are more appealing to today's consumers. *Boerhavia*, a genus of Nyctaginaceae comprising approximately 40 species, is mainly found in tropical and subtropical regions, particularly on gravel plains and rocky slopes (Spellenberg, 2012). In particular, one of the most species in the genus Boerhavia is *Boerhavia elegans Choisy* (Alhydwan), a plant native to Asia and Africa that was described and documented in detail by Fosberg in 1978. Alhydwan has been a key part of indigenous and tribal cuisine for generations, used in making porridge, desserts, and savory dishes. It's often enjoyed mixed with bread and cakes, adding a delightful flavor to the dishes (Al-Farga *et al.*, 2014).

Study objectives:

This study aimed to investigate the differences between aqueous and ethanolic extracts of Al-Haydawan in terms of mineral, antioxidant, and phenolic content.

2- Materials and Methods:

Preparation of alhydawan extract:

Al-Haydawan seeds were purchased from the local market of Al Madeena and Sukkari date powder was purchased from a local shop in the local market of Qassim. The seeds were carefully washed to remove impurities and then finely crushed and ground in a mortar. The ground seeds were split into two identical sections. The initial 100 grams were immersed in a 70% ethanol solution at a ratio of 1:4, then stored at a temperature of 5 ± 2 °C for three days. The resulting extract was centrifuged at 5000 × g for 30 min and the process was carried out two times. The supernatant was gathered and subjected to evaporation using a rotary vacuum evaporator set to 50°C (Penna et al., 2003). The second portion, weighing 100 g, was immersed in 800 ml of hot water at 80 °C for one hour. After soaking, it was allowed to cool to room temperature and stored at a temperature of 5 ± 2 °C for three days. The study was conducted at the Department of Food Science and Human Nutrition, Faculty of Agriculture and Nutrition, Qassim University.

Phytochemicals and Antioxidant Activity

Total Antioxidant Activity:

FRSA evaluation was performed according to the method of Lee *et al.*, (1998). An aliquot (1 mL) of the methanol extract was mixed with the DPPH solution (2 mL). Equal volumes of methanol and DPPH were used as controls. The wavelength of the spectrophotometer was 518. The percentage inhibition was calculated as follows:

$$\text{Antioxidant Activity (\%)} = 1 - \frac{A1}{A0} \times 100$$

The absorbance of the control extract is represented by (A0), while (A1) denotes the absorbance of the sample extract.

Determination of Total Phenolic Contents (TPC):

The TPC value of the extract prepared in each sample was determined using Folin-Ciocalteu reagent according to the method of Bettaieb *et al.* (2010). The measured results were compared with the gallic acid solution (GA) standard curve (R2 = 0.99), and the TPC content per gram dry weight (DW) was expressed as milligram gallic acid equivalent (mg GAE g−1 DW).

Minerals analysis

Minerals content of the samples were done using an Optima 4300 DV inductively coupled plasma atomic emission spectroscopy (ICP-OES, Perkin Elmer, MA, USA) as described previously (Milani *et al.*, 2018). Briefly, 0.5 g sample will be taken in glass flasks. 5 mL of H_2SO_4 was added and samples were placed on hotplate for 1 hour at 450 ^{O}C. samples were heated until fumes were exhausted. Samples were removed from the hotplate and let them cool. After that 3mL H_2O_2 was added and samples were placed on hotplate until clear solution. At the end, samples were removed from hotplate and let them cool. The solution was filtered by Whatmann 42 filter paper and filtrate was diluted up to 50 mL final volume. Latterly samples were analyzed by ICP-OES (PerkinElmer Optima 4300 DV ICP-OES).

Statistical analysis:

Statistical data analysis was performed using SPSS, with three replicates for each treatment. The data obtained was analyzed using one-way ANOVA. The comparison between the average coefficients using Duncan's Multiple Range Test is at a significance level of p < 0.05. (SPSS Inc., NY, and USA).

3- Results

Table 1. DPPH and TPC in two extracts during the storage period

Parameters	Storage period (days)	treatments	
		Water extract	Ethanolic extract
TPC (mg GAE/kg)	1	12.47 ±0.04 ^{Bb}	22.48 ±0.11 ^{Aa}
	7	13.85 ±0.09 ^{Ba}	19.81 ±0.09 ^{Ab}
DPPH (%inhibition)	1	96.42 ±0.04 ^{Ba}	129.44±0.12 ^{Aa}
	7	94.65 ±0.07 ^{Ab}	124.84±0.06 ^{Bb}

Means (n = 3, ±SD) with the same small letters in the same column and capital letter in the same raw are not significantly different (p < 0.05)

The highest levels of phenols in Al-Haydawan plant were found in its ethanolic extract. On the first day of testing, the total phenol content reached 22.48 mg GAE/kg, which decreased to 19.81mg GAE/kg by the seventh day of refrigeration. Additionally, the water aqueous measured 12.47 on the first day and increased to 13.85 mg GAE/kg on the seventh day (**Table 1**). Antioxidants were found to be most concentrated in the alcoholic extract, with a peak value of 129.44% on the first day. By the seventh day of storage, this value had slightly decreased to 124.84%. In contrast, the aqueous extract showed values of 96.42% and 94.65% on the first and seventh days, respectively. Antioxidants play a crucial role in food preservation, which is why researchers are eager to discover efficient extraction methods for these valuable molecules. A well-known technique for extraction utilizes various solvents, including water, ethanol, methanol, and hexane (Santos & Gonçalves, 2016). The success of extracting phenolic compounds largely depends on factors like the type of solvent used, its concentration, and the exposure time (Azmir *et al.*, 2013). However, some solvents are restricted due to their toxic properties, as governed by regulations. Ethanol, on the other hand, serves as a safe alternative for separating compounds intended for human consumption (Ignat *et al.*, 2011).

Table No. 2 reveals notable differences in the microelement values between the two extracts. On the first day of extraction, the alcoholic extract recorded copper, iron, and zinc values of 177.8, 7.24, and 3.81, respectively. In contrast, the aqueous extract showed values of 141.8, 4.12, and 3.79 for the same elements.

Table 2. Micro-element content (mg/kg) of two extracts during the storage period

Parameters	Storage period (days)	Water Extract	Ethanolic Extract
Cu	1	141.8±0.1^{Bb}	177.8±0.17^{Ab}
	7	147.3±0.2^{Ba}	186.7±0.24^{Aa}
Fe	1	4.12±0.12^{Bb}	7.24±0.99^{Ab}
	7	4.54±0.74^{Ba}	7.55±0.78^{Aa}
Mn	1	1.32±0.4^{Bb}	1.52±0.46^{Ab}
	7	1.47±0.5^{Ba}	1.68±0.52^{Aa}
Zn	1	3.79±1.09^{Aa}	3.81±0.01^{Aa}
	7	3.11±0.21^{Bb}	3.81±0.01^{Aa}
B	1	3.01±0.01^{Ba}	4.19±0.76^{Ab}
	7	3.04±0.01^{Ba}	5.24±1.09^{Aa}
Cd	1	0.24±0.03^{Ba}	0.34±0.09^{Ab}
	7	0.21±0.02^{Ba}	0.41±0.02^{Aa}

Means (n = 3, ±SD) with the same small letters in the same column and capital letters in the same raw are not significantly different ($p < 0.05$)

Table 3. Macro-elements (mg/kg)) of two extracts during the storage period

Parameters	Storage period (days)	Water Extract	Ethanolic Extract
Ca	1	65.08±1.88^{Bb}	70.89±1.87^{Ab}
	7	73.34±1.03^{Ba}	78.74±1.04^{Aa}
K	1	172.11±3.24^{Aa}	168.91±1.21^{Ba}
	7	156.8.±3.42^{Bb}	162.0±3.12^{Ab}
Na	1	55.11±1.83^{Bb}	79.31±2.13^{Aa}
	7	59.25±1.23^{Ba}	67.9±3.76^{Ab}
P	1	32.24±1.2^{Ba}	52.24±1.233^{Ab}
	7	31.82±1.21^{Ba}	56.7±1.24^{Aa}
Mg	1	11.05±2.76^{Ab}	11.24±2.76^{Ab}
	7	14.13±3.42^{Aa}	13.69±3.02^{Aa}

Means (n = 3, ±SD) with the same small letters in the same column and capital letters in the same raw are not significantly different ($p < 0.05$)

Minerals have important functions in the metabolic and physiological processes of living organisms. Trace elements are important compounds required in small amounts for biochemical reactions and to maintain human health. **Table 3** shows that macro minerals are most concentrated in the alcoholic extracts. In particular, the Calcium content showed significant values, reaching 70.89 mg/kg on the first day of storage and 78.74 mg/kg on the seventh day. In contrast, the Calcium value in the aqueous solution was initially 65.08 mg/kg and increased to 73.34 mg/kg on the seventh day. As shown in the table, the sodium and potassium values in the alcoholic extract were 79.31 mg/kg and 168.91 mg/kg, respectively. On the first day of extraction, the sodium and potassium values in the aqueous solution were 55.11 and 32.24, respectively. On the seventh day, the potassium value in the alcoholic extract increased to 56.7 mg/kg, while the sodium value decreased to 67.9 mg/kg. In a previous study by Farga *et al.*, (2016), it was found that the seeds are an important natural source of calcium and potassium and have a higher mineral content compared to other sources.

Discussion

Solvent extraction is a common method for isolating antioxidants from plants. It is critical to choose a solvent that matches the polarity of the antioxidant. This step is very important to enhance the efficiency of compound extraction from the plant (Rajan *et al.*, 2020).

Al-Farga *et al.* (2018) investigated how different polar solvents affect the phenolic content and antioxidant activity of Alhydwan (*B. elegana choisy*) seed flour. The solvents tested included methanol, ethanol, acetone, diethyl ether, ethyl acetate, and hexane. They evaluated the antioxidant properties of the seed flour extracts in comparison to butylated hydroxytoluene (BHT), which served as a positive control. The findings indicated that The choice of solvent notably affected the extraction yield, the concentration of total phenolic, antioxidant activity in Alhydwan seed flour. Methanol proved to be the most effective solvent, yielding higher amounts of phenolic compounds and showing better antioxidant activity. These results suggest that the characteristics of extraction solvents are crucial for effectively extracting phenolic compounds and enhancing antioxidant activity.

The food industry has seen an increasing incorporation of plants and their extracts, known for their beneficial and aromatic compounds. These natural ingredients are utilized for various purposes, including flavoring, coloring, acting as antioxidants, serving as antimicrobial agents, boosting nutrients, providing enzymes, and enhancing packaging (Mahunu *et al.*, 2021; Rashmi and Negi, 2022). Multiple factors influence the concentration of chemical compounds in plants. Key variables include soil quality, climate conditions, farming practices, the water used for irrigation, plant handling, harvest timing, and environmental pollution (Aldhanhani, 2022; Caldeirão *et al.*, 2021). To ensure consumer safety, it's crucial to prevent harvesting plants from areas with high levels of industrial and transportation activities, as these could lead to heavy metal contamination (Ahmed, *et al.*, 2021; Luo *et al.*, 2021).

Furthermore, following proper agricultural practices while utilizing chemical fertilizers or pesticides is crucial. Before extraction, plant material typically goes through pre-treatment such as washing, drying, and enzymatic processes. According to Ahmed *et al.*, (2021), washing treatment can slightly decrease the occurrence of aluminum and lead heavy metal pollutants in plant samples.

Al-Farga *et al.*, (2014) conducted a study and found that ethanol extracts from *B. elegana* seeds showed higher levels of phenolic content. The quantification of phenolic content was measured in Gallic acid equivalent, resulting in a value of 253.9 ± 0.9 mg/g. The presence of phenolic compounds in this plant is significant due to their valuable pharmacological effects. Sadeghi *et al.*, (2015) conducted a study in which they examined the antioxidant efficacy of various extracts from different parts of the *Boerhavia elegana* plant. The results showed that the methanolic extract demonstrated the highest activity in both the (DPPH) and ferric reduction activity potential**.** Medicinal plants have long been recognized as a vital source of natural antioxidants, especially phenolic compounds, vitamins, carotenoids, and essential metals like copper, iron, manganese, and zinc (Butorová *et al.*, 2017). Their components are commonly utilized not only to enhance food quality but also in the of functional foods formulation.

Conclusion

The selection of appropriate solvents during the extraction process is crucial, as it significantly impacts both the properties and the yield of secondary metabolites derived from medicinal plants. Furthermore, raising cultural and health awareness about the benefits of functional foods is vital at different stages of life, considering their important role in maintaining health, preventing diseases, and supporting therapeutic practices.

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