Journal of Food Measurement and Characterization (2018) 12:2121–2127
https://doi.org/10.1007/s11694-018-9827-z

ORIGINAL PAPER

Solvent effects on antioxidant activities and phenolic contents of the alhydwan (*Boerhavia elegana choisy*) seed flour

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Received: 28 November 2017 / Accepted: 7 May 2018 / Published online: 18 May 2018

Abstract
The effects of using solvents with different polarities on the yields of extracting phenolic compounds and antioxidant activity of novel food Alhydwan seed flour were investigated. The extraction solvents used include methanol, ethanol, acetone, diethyl ether, ethyl acetate and hexane. Results showed the efficiencies of the solvents in the extraction of phenolic compounds and antioxidant activities were in this order: methanolic extract < ethanolic extract < acetone extract < diethyl ether extract < ethyl acetate extract < hexane extract. The phenolic content was 63.82–177.08 mg of gallic acid equivalents/g and the yield extract ranged as low as 8.49–126.10%. The results have shown that the properties of extraction solvents significantly affected extraction yield, total phenolic compounds and antioxidant activity of the Alhydwan seed extract. Furthermore, results showed that the methanol extraction resulted in the highest yield (80%), higher phenolic compounds and better antioxidant activity compared to the other solvents.

Keywords Antioxidant activity · Alhydwan (*Boerhavia elegana choisy*) seed flour · Extraction solvent · Phenolic compounds

Introduction

Oxidation is the main cause of damage and corruption oils that cause the emergence of off flavors (rancidity) causing a reduction in the validity period and the nutritional value and not the possibility of oil for human consumption [1, 2]. Industrially produced antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tetra butylated hydroxy quinon (TBHQ) have been added to food to counteract oxidation. However, there has been a growing concern about the possibility of their use in food production because of the presence of many of the statements and reports about its impact on the health of the consumer in terms of their ability to form toxic and carcinogenic compounds linked to liver and kidney disease [3]. Therefore, to prevent the use of some industrial antioxidants, especially (TBHQ) in industrial food processing in all of Japan, Canada and the countries of the European common market, and also to exclude (BHA) from the list of materials that are commonly used [4]. This has led to the existence of a growing trend to replace the industrial antioxidants with naturally produced antioxidants such as catechins which are deemed to be safer in terms of health and nutrition, particularly botanically sourced either from herbs or leaves or fruits or seeds or roots or tubers or even of secondary waste for Food processing plants [5, 6]. Recently, there has been an increase in the use of plants and herbs as antioxidants in processed foods as an alternative to synthetic antioxidants [7]. The reports of ill effects of synthetic chemicals found in foods has led to increased awareness among consumers thus, increased demand for non-toxic, natural preservatives [8]. It has been reported that extracts from herbs and spices

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have been used to evaluate antioxidants and antimicrobial properties [9]. It has also been reported that the extraction of polyphenols from plant using different solvent systems and the rate of return mainly depends on the method of extraction and solvent [10, 11]. It is worth mentioning that the extraction method must ensure their chemical modification and it must allow complete extraction of the compounds of benefit [12]. It has been reported that a higher content of polyphenols was dependent on the solvents and a higher content of polyphenols was obtained by an increase in the polarity of the solvent used [8]. There are several studies that have been used mixtures of ethanol, methanol, acetone, absolute methanol, absolute ethanol and water, to extract of polyphenols from plants [6–14]. A study by Wang and Helliwell [15] reported that the use of ethanol was superior to aqueous methanol and acetone for extraction of the flavonoids from tea while in another study [16] it was reported that water was the best solvent for extracting tea catechins compared with 80% methanol and 70% ethanol. Similarly, it was reported that a substantial amounts of antioxidants were found in extracts from wheat by using various solvents such as (methanol, ethanol, aqueous ethanol solution and water) [17, 18]. *Boerhavia elegana choisy* seeds (common name: alhydwan) is an edible herbaceous member of the Nynctaginaceae family commonly found in Southern Yemen [19]. It has a long history of usage by indigenous and tribal people in the making of traditional cuisines and as one of the staple ingredients in the manufacture of porridge, desserts and savory products. Alhydwan is also consumed as a food supplement in bread and cakes where it is characterized by a good flavor [20]. The alhydwan seed flour contains high composition of dietary fiber (36.13%), protein (14.60%), ash (6.88%), and fat (11.49%) [21]. It is high in unsaturated fatty acids (74.6% of total fatty acids), and low in saturated fatty acids (22.2%); linoleic acid constitutes is about 12.9% of total fatty acids, Oleic acid about 57.8% [21]. However, up to this date, as far as our knowledge is concerned, there is no literature about this seed. The objective of this current study was therefore to investigate the effects of using different extracting solvents (methanol, ethanol, acetone, diethyl ether, ethyl acetate and hexane) on total polyphenol and antioxidant activity of a novel food: Alhydwan (*B. elegana choisy*) seed flour.

Materials and methods

Materials and chemicals used

Dried alhydwan seeds as shown in Fig. 1. were collected in the month of June, 2014 from a farmland in Hadramout city, Yemen, and transferred to the Department of cereal Chemistry, Faculty of Engineering and Technology, Jiangnan University, Wuxi City, People's Republic of China. All reagents used in the experiments were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO). Solvents used for extraction of Alhydwan (*B. elegana choisy*) seed flour (Methanol and Ethanol, Acetone, Diethyl ether, Ethyl acetate, Hexane) were purchased from J&K Scientific Ltd (Beijing, China).

Fig. 1 Alhydwan seed (*B. elegana choisy*)

Preparation of plant extracts

Preparation of plant extracts the dried alhydwan seeds were ground (Model FW100 grinder, Tianjin Taisite Instrument Co., Ltd., Tianjin, China) to produce a fine powder that would pass through a 80 mesh sieve. The ground powders were placed in plastic bags, tightly closed, and stored at − 20 °C until use. The extraction process involved mixing one part of the extraction powder with three parts of the solvents (methanol 80%, ethanol 80%, acetone, diethyl ether, ethyl acetate and hexane). Extraction was done by the Soxhlet apparatus for a period of 8 h and the temperature used was, according to the degree of evaporation of the solvent used. The solvents were then removed using a rotary evaporator (Model RV10 basic, Guangzhou IKA Scientific Instrument Co., Ltd., Guangzhou, China) under reduced pressure at 40 °C. Then dried extract was freeze dried to obtain the crude extract dried powder.

Extraction of alhydwan flour polyphenols

The polyphenols from the alhydwan flour sample (0.2 g) were extracted using either organic solvents or distilled water. For the water extraction method, alhydwan flour was soaked with 10 ml freshly boiled distilled water for 10 min in a thermos flask. The infusion was filtered through Whatman No. 1 and rapidly cooled under tap water. For organic solvent extraction, methanol 80%, ethanol 80%, Acetone, Diethyl ether, Ethyl acetate and Hexane were used. Our results from the preliminary work revealed that methanol extraction of alhydwan resulted in higher polyphenol content compared

with other solvents. Ground alhydwan sample (0.2 g) was extracted with 2 ml of solvent for 1 h on a horizontal shaker. The mixture was centrifuged at 8500×*g* for 10 min and subsequently decanted. The residue was re-extracted twice for 2 h and the extraction procedure was repeated twice more for 3 h as explained above. The five supernatants were combined and stored at − 18 °C prior to analysis. Each solvent extraction was carried out in triplicate.

Total phenolic content

Total phenol content of plant extracts of alhydwan seed flour was estimated using Folin–Ciocalteu reagent (FCR) by the method of [22] with some modifications. (7.5) ml of FCR which was diluted 10-fold with distilled water mixed with 1 mili liter of each plant extract solution, which was prepared with ethanol at a concentration of 0.1 mg/ml, then added with 7.5 ml of 60 mg/ml of aqueous Na_2CO_3 solution after standing at room temperature for 5 min. Absorbance was measured at 725 nm for the mixture after 2 h of keeping at room temperature. The results were expressed in Gallic acid equivalents (GAE), determined by utilizing a separately prepared absorbance versus concentration curve for Gallic acid.

Measurement of reducing power

The reducing power of the extracts was determined according to the procedure described earlier [23], with a slight modification. A 2.5 ml fraction of alhydwan seed flour was mixed with 2.5 ml of 1% potassium ferricyanide and 2.5 ml of phosphate buffer (200 mM, pH 6.6); the mixture was incubated at 50 °C for 20 min. Then 10% trichloroacetic acid (5 ml) was added and the mixture centrifuged at 980 *g* for 10 min at 5 °C in a refrigerated centrifuge (CHM-17; Kokusan Denki, Tokyo, Japan). The upper layer of the solution (5.0 ml) was decanted and diluted with 5.0 mL of distilled water and ferric chloride (1.0 ml, 0.1%), and absorbance read at 700 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). All samples were analyzed thrice and the results averaged.

Hydrogen peroxide-scavenging activity

The ability of the extracts to scavenge hydrogen peroxide was estimated by the method of [24]. A solution of hydrogen peroxide (2 mM) was prepared in 50 mM phosphate buffer (pH 7.4). The molar extinction coefficient for H_2O_2 of 81/mol cm was used to determine the concentration of hydrogen peroxide spectrally in the absorption of 230 nm. The following was placed in a test tube: gallic acid or BHT (0.31–1.25 mg/ml), aliquots (0.1 ml) of water (0.625–5 mg/ml), infusion (0.625–5 mg/ml), ethanol (0.31–3.75 mg/ml) and ethyl acetate (1.25–5 mg/ml) extracts, and their volumes were made up to 0.4 ml with 50 mM phosphate buffer (pH 7.4) or solvents. Then added 0.6 ml hydrogen peroxide solution, tubes were vortexed and absorbance was measured of the hydrogen peroxide at 230 nm after 10 min, against a blank solution containing 50 mM phosphate buffer without hydrogen peroxide.

The abilities to scavenge the hydrogen peroxide were calculated according to the following equation:

$$\text{Hydrogen peroxide scavenging activity (\%)} = (1 - \text{Absorbance of sample} / \text{Absorbance of control (BHT)} \times 100)$$

Linoleic oxidation scavenging activity

Antioxidant activity was also estimated using the Ferric thiocyanate (FTC) test by the method of [25]. The absorption of the resulting mixture of the color red was measured at 500 nm every 24 h until the absorbance of the control reached its maximum, for 3 days. BHT was used as a positive control, whereas water was used as the negative control.

Determination of antioxidation activity by β-carotene bleaching method

Antioxidant activity was estimated also using the β-carotene/linoleic acid emulsion test by the method of [26]. An amount of 2 mg of β-carotene were dissolved in 10 ml of chloroform and 1 ml b-carotene solution was mixed with 20 mg of purified linoleic acid and placed into a round-bottom flask containing 200 mg Tween 40 emulsifier. The chloroform was removed by a rotary vacuum evaporator, distilled water (50 ml) was added to the flask and the mixture was stirred in a sonicator. Alhydwan seed flour extract (2 mg/ml) or the synthetic antioxidant (BHT), was added to 5 ml of the β-carotene/linoleic acid emulsion and tested with two final concentrations of 100 and 200 mg/l. 5 ml β-carotene/linoleic acid emulsion and 0.2 ml water(control). Absorbance at 470 nm was recorded straightway after the addition of the sample to the emulsion, which was regarded as t = 0 min. The bottles were covered and placed in a water bath at 50 °C. The second emulsion (B) was prepared, which consisted of 50 ml of water, 100 mg of Tween 40 and 20 mg of linoleic acid. Absorbance was determined for emulsion at 470 NM every 15 min until 120 min. Water (200 µl) with 5 ml emulsion B was used to zero the spectrophotometer. Coefficient antioxidant activity (AAC) was calculated according to the following equation:

$$\text{AAC} = \left(A_{A(120)} - A_{C(120)}\right) / \left(A_{C(0)} - A_{C(120)}\right) \times 100$$

where $A_{A(120)}$ is the absorbance of the antioxidant at 120 min, $A_{C(120)}$ is the absorbance of the control at 120 min, and $A_{C(0)}$ is the absorbance of the control at 0 min.

Statistical analysis

All experiments were conducted in triplicate and statistical analysis was performed using test SAS [27]. Duncan's test was used to perform multiple comparisons among means at $p < 0.05$ significance level.

Results and discussions

Extraction percentage of the phenolic compounds

Extraction yields

Results for the average percentage values of yields for different extracts obtained by different solvents are presented in Table 1. From the results, it is evidently clear that the highest yields were recorded by methanol extraction compared to the other solvents namely ethanol, acetone, diethyl ether, ethyl acetate and hexane. When computed in terms of percentage, methanol extraction registered 26% based on dry weight of extract while the percentages of ethanol, acetone, diethyl ether, ethyl acetate and hexane were 21.70, 18.74, 14.09, 12.83 and 8.49% (dry weight) respectively. The differences in the yield extract using the different solvents have been attributed to the differences in polarities of different compounds present in seeds and such differences have been reported in the literature relating to the fruit seeds [28]. This difference has subsequently led to the differences in the solubility of the solvent used where the yield percentage extracted depends on the type of solvent and therefore this meant that the solvent with high polarity could be able to extract the most polar compounds [29–35]. Our results have revealed that the yields obtained by using solvent with high polarity as opposed to a solvent with less polarity is what distinguishes the phenolic compounds which are polar in their nature because they contain hydroxyl groups and have the ability to correlate with hydrogen with solvents which are more polarity [30]. The order of the extracts, obtained in this study from the highest was: methanol 1 > ethanol 2 > acetone 3 > diethyl ether 4 > ethyl acetate 5 > hexane 6.

Table 1 Yield extract and total phenol content of dried alhydwan seeds obtained by using different solvents

Solvent type	Yield extract (%)	Total phenol content (mg) gallic acid/g dried extract
Methanol	26.10[a]	177.08[a]
Ethanol	21.72[b]	161.52[b]
Acetone	18.74[c]	138.35[c]
Diethyl ether	14.09[d]	105.11[d]
Ethyl acetate	12.81[e]	94.57[e]
Hexane	8.49[f]	63.82[f]

Mean of three determination SD. Mean values in a column with different letters are significantly ($p < 0.05$) by Duncan's multiple range test

Amount of total phenolics

Results for the total amount of phenolics extracted from the crude alhydwan dried seeds using the different solvents are shown in Table 1. The amounts extracted using methanol, ethanol, acetone, diethyl ether, ethyl acetate and hexane are 177.08, 161.52, 138.35, 105.11, 94.57, 63.82 respectively expressed as mg GAE/g of dry material. From the results, the amounts of total phenolics of dried alhydwan seed extracted with methanol was significantly higher ($p < 0.05$) compared with the other solvents and as previously stated this has been attributed to the fact that solvents with high polarity are efficient in extracting phenolic compounds and in this case methanol is more polar than the other solvents [31]. It has also been reported that [32] the most polar solvents such as methanol have ability to extract more phenolic compounds compared with less polar solvents such as hexane and chloroform. The results shown in this study that the higher content of polyphenols was obtained by using solvent with high polarity as compared with solvents with less polarity was basically dependent on the type of solvents. These findings are in agreement with [8] who found that higher content of polyphenols extracted was dependent on the solvents and a higher content of polyphenols was obtained with an increase in the polarity of the solvent used. The highest amount of polyphenol content extracted in this study was found when methanol was used and the order was as follows: methanol 1 > ethanol 2 > acetone 3 > diethyl ether 4 > ethyl acetate 5 > hexane 6. In all the cases, hexane was the least effective solvent.

Antioxidant activity

Taking into consideration the differences among several of the systems available, it has been reported that the results of a single method can give only a reductive suggestion of the antioxidant properties of the extracts [33]. Bearing this in mind, we used four assays in this present study, namely reducing power, based on bleaching of β-carotene, hydrogen peroxide hydrolysis scavenging and linoleic oxidation scavenging activity.

Reducing power

Results for the reduction power values (expressed in shorthand absorption at a wavelength of 700 nm) crude extracts of the dried seed flour alhydwan in different solvents at concentrations of 0.2, 0.4, 0.6, 0.8, 1 (mg/g) are presented in Fig. 2. Results showed significantly higher values ($p < 0.05$) of absorption crude extracts of dried alhydwan seed extracted

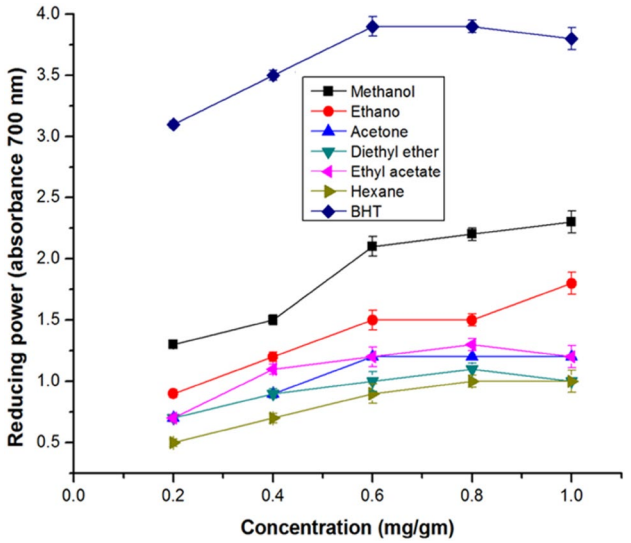

Fig. 2 Reducing power (absorbance 700 nm) for the raw extracts of dried alhydwan seeds. All determinations were carried out in triplicates and mean value ± standard deviation. Mean values are significantly different ($p < 0.05$)

by methanol compared with values obtained using the other solvents where the percentage reached by methanol extract was 2.3 mg/g while percentages reached for ethanol, acetone, diethyl ether, ethyl acetate and hexane were 1.8, 1.2, 1.2, 1.0 and 1.0 mg/g respectively. It has been reported that an increase solvent polarity can result in increased amounts of extracted phenolic compounds [29] and this has been demonstrated by the findings in this study. As shown in Fig. 2, an increase in the concentration of extracts using different solvents led to a significant increase ($p \leq 0.05$) in the absorbance values (higher reducing power), where absorbance values reached for the samples extracted with methanol, ethanol, acetone, diethyl ether, ethyl acetate and hexane at concentration of 0.2 mg/ml 1.3, 0.9, 0.7, 0.7, 0.7, 0.5, respectively, and at concentration 1 mg/ml were 2.3, 1.8, 1.2, 1.0, 1.2, 1.0, respectively. These findings suggest that the strength of shorthand for the samples studied depends on the concentration of the extracts, and this explains the presence of compounds reductones reductionist in higher quantities as in the case of use of the highest concentrations the extract and this is in agreement with [34] who reported on the antioxidant activity of the extracts of some Malaysian plants and showed that the increase of the concentration of the extract led to increased absorption values, thus, granting the highest reducing power (effective antioxidant higher). However, the values of reducing power of alhydwan seed for all the samples were less compared to the control sample (BHT). The order of the values for the reducing power obtained in this study was as follows: methanol 1 > ethanol 2 > acetone 3 > diethyl ether 4 > ethyl acetate 5 > hexane 6.

Hydrogen peroxide-scavenging activity

The results shown in Fig. 3 showed portability crude extracts of alhydwan seed using different solvents ability to inhibit the decomposition of hydrogen peroxide. The results have revealed that regardless of solvent type and concentration, all the samples have the ability to curb decomposition of hydrogen peroxide. Furthermore, methanol has shown to have significantly higher ($p < 0.05$) ability in curbing decomposition of hydrogen peroxide compared to the other solvents. The results suggest that an increase in the concentration of the extracts which subsequently results in increasing percentages have a high ability in curbing decomposition of hydrogen peroxide. These findings are in agreement with the results of other authors who have reported that a higher extract concentration translates to higher antioxidants content resulting in high efficiency in curbing the decomposition of hydrogen peroxide as it possess hydroxyl making it a strong oxidizing agent [33]. The results have also shown that the control sample (BHT) registered a higher value in decomposition of hydrogen peroxide compared to the values obtained by the other solvents. The differences in the results might be attributed to the fact that the crude extracts of alhydwan seed might have contained a mixture of both phenolic compounds and non-phenolic which led to some interference as compared in the scenario where BHT was used.

Linoleic oxidation scavenging activity

Results for the linoleic oxidation scavenging activity of crude extracts of alhdwan seed in presence of ferric

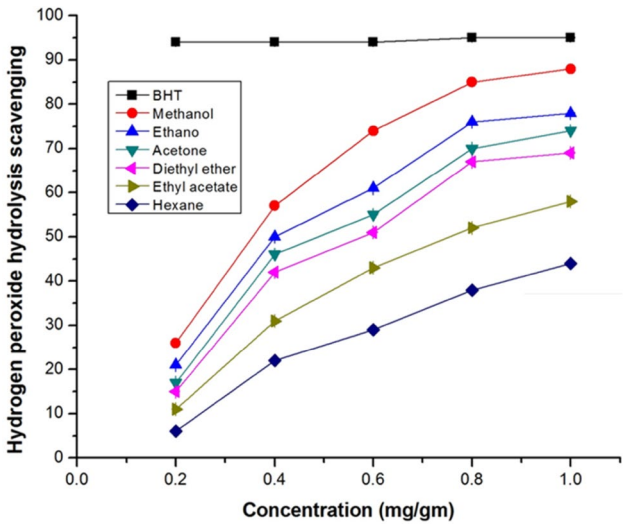

Fig. 3 Hydrogen peroxide hydrolysis scavenging of dried crud alhydwan seeds. All determinations were carried out in triplicates and mean value ± standard deviation. Mean values are significantly different ($p < 0.05$)

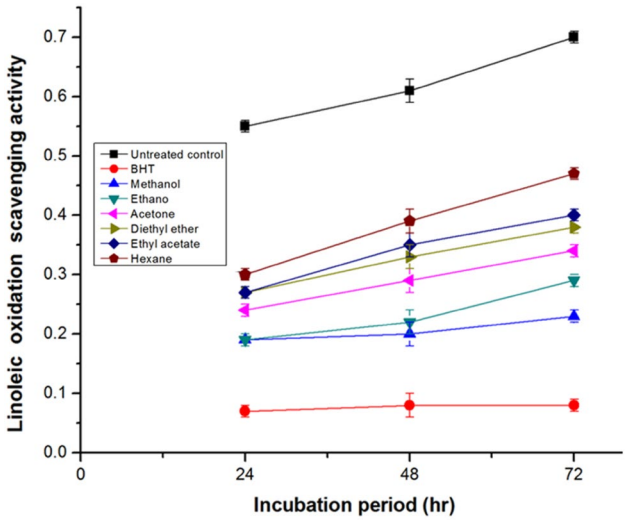

Fig. 4 Linoleic oxidation scavenging activity (absorbance 500 nm) of dried crude alhydwan seeds extracts (0.02 mg/ml) by F.T.C. assay. All determinations were carried out in triplicates and mean value ± standard deviation. Mean values are significantly different ($p < 0.05$)

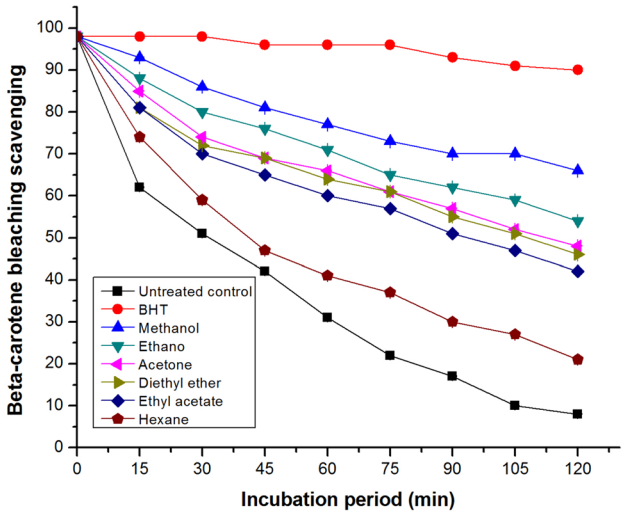

Fig. 5 β-carotene bleaching scavenging of dried crud alhydwan seeds extracts

thiocyanate using different solvents are shown in Fig. 4. The absorption of the colour of the treated extract sample was measured at wavelength of 500 nm. Results show that the control sample (BHT) registered lower absorption values as compared to the values obtained by using the other different types of solvents. These differences in the results are attributed to the previously stated reasons of the extracts having a mixture of both antioxidant and non antioxidant materials. Furthermore, the results have shown that apart from the control (BHT), values for methanol were significantly lower ($p < 0.05$) compared to the other solvents. These findings are in agreement with other authors [5] in a study focusing on the effectiveness of anti-oxidation of some secondary waste of the food plants and [34] in a study looking at the effectiveness of anti-oxidation for fruit Rhus.

Percent β-Carotene bleaching

Results as presented in Fig. 5 shows the crude extracts from alhydwan seed using different solvents ability in curbing pigment beta-carotene by oxidation of the products of the linoleic acid. The results showed a decreasing trend in the percentages for the effectiveness of antioxidant in both the control sample as well as the other solvents at concentration 0.2 mg/ml during the incubation periods 0, 15, 30, 45, 60, 90, and 120 min. However, from the results it is very clear that there was a stable small decrease in the percentages for the effectiveness of the sample control (BHT) as compared to the other solvents. This demonstrated that that control sample (BHT) had effective antioxidant ability and this is attributed to the fact that the crude extracts from alhydwan and the other solvent treatments had mixtures of compounds which might have overlapped with antioxidant compounds such as phenolic compounds thereby reducing their impacts. In addition, BHT has been reported to be a pure phenolic compound thereby having a high effectiveness when used as antioxidants [35]. Other authors [29] have reported differences in the effectiveness of antioxidants using extracts and solvents and found out that methanol treated samples were significantly effective against oxidative stress. This is consistent with the results of our study where methanol crude extract from alhydwan had significantly higher phenolic compounds compared with extracts of the other solvents.

Conclusion

In this study, the effects of using solvents with different polarities on the phenolic contents extracted and the antioxidant activities of novel food Alhydwan (*B. elegana choisy*) seed flour were investigated. The solvents used included methanol, ethanol, acetone, diethyl ether, ethyl acetate and hexane. Additionally, the antioxidant activities of Alhydwan (*B. elegana choisy*) seed flour extracts were evaluated and compared with butylated hydroxy toluene (BHT) as a positive control compared sample. Results showed that properties of the extraction solvents significantly affected extraction yield, total phenolic compounds and antioxidant activity of Alhydwan (*B. elegana choisy*). The results have revealed that methanol registered a high extraction yield, high phenolic compounds and a better antioxidant activity. It can therefore be concluded that properties of extraction solvents plays a significant contribution in determining the

effectiveness of solvents in phenolic compounds extraction and antioxidant activity.

Acknowledgements The authors would like to acknowledge the Priority Academic Program Development of Jiangsu, Higher Education Institutions, Wuxi city, Jiangsu province, People's Republic of China. We are also grateful to all the staff and students of the Research Centre of Functional Ingredients and Healthy Foods laboratory, and who helped us to bring the raw materials to China.

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