

## EFFECT OF SOLVENT TYPE ON THE EXTRACTED BIOACTIVE COMPOUNDS OF MALLOW (*MALVA SYLVESTRIS*) LEAVE

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**ABSTRACT:** *The effect of different extraction solvents (water, 70% ethanol and 95% methanol) on bioactive compounds (polyphenols, flavonoids and saponin and tanins) and antioxidant characteristics of mallow leaves extracts were investigated. The total phenolic compounds extracted from green parts of mallow using 70% ethanol, 95% methanol and water were 57.2, 43.2 and 121.6 mg/g, respectively. The total flavonoids content of mallow water extract is significantly ( $p \leq 0.05$ ) higher than those of methanolic and ethanolic extracts. Tannins content in mallow extract obtained by 70% ethanol was 15.52 mg tannic/g which was significantly higher than the other solvents, meanwhile the lowest amount (4.9 mg tannic/g) was detected in 95% methanolic extract. The same trend was also found for saponin, since the highest value (6.7 mg/g) was in 70% ethanolic extract. Total antioxidant capacity (TAC) of mallow extracts at 500 ppm decreased as the percentage of alcohol in the solvent increased. Whereas, the highest TAC ( $58.7 \pm 1$  %) was detected in water extract and decreased gradually by decreasing the percentage of water in the solvent. The water extract at 500 ppm had significantly higher ( $p \leq 0.05$ ) antioxidant activity compared to the other solvents. Consequently, the water was the best solvent for extraction of phenolics and flavonoids from green parts of mallow and these extracts have a strong antioxidant activity.*

**Key words:** *Mallow extract, antioxidants, phenolic, DPPH*

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### INTRODUCTION

*Malva sylvestris* is a species of the genus *Malva* in the family of *Malvaceae* and is considered to be the type species for the genus. It is known as common mallow or mallow to English speaking (Milin *et al.*, 2003 and Yeole *et al.*, 2010). Plant polyphenols are aromatic hydroxylated compounds, commonly found in vegetables, fruits and many food sources that form a significant portion of our diet, and which are among the most potent and therapeutically useful bioactive substances. Phenolic derivatives represent the largest group known as 'secondary plant products' synthesized by higher plants, probably as a result of antioxidative strategies adapted in evolution by respirative organisms starting from precursors of cyanobacteria (Bennick, 2002). Mallow extracts are reported for their radical

scavenging effect (Karakaya, 2004). Different reports in the literatures displayed that extracts of either edible or non-edible plant materials have antioxidant effect owing to the presence of some active phytochemicals, especially, phenolic compounds (Lako *et al.*, 2007; Pellegrini *et al.*, 2007; Benavente *et al.*, 2000; Emmons and Peterson, 1999). On the other hand, plant material wastes from food industries involve high which has an adverse environmental impact. Thus, considerable importance on the recovery, recycling and upgrading of food industry wastes to decrease the environmental pollution (Laufenberg *et al.*, 2003; Reddy and Yang, 2005). Mallow can be considered as good sources of some phenolic and antioxidant compounds (Beghdad *et al.*, 2014). *Malva sylvestris* L. is one of the most promising medicinal plant species. However, extensive

research in the area of isolation and characterization of the active compounds of *M. sylvestris* is essential so that better, safer, and cost-effective drugs for curing various diseases and infections can be developed (Paul, 2016). The objective of this work was to evaluate the effect of different extraction solvents on the yield, bioactive compounds and antioxidant properties of mallow.

## **MATERIAL AND METHODS**

### **1. Materials**

The green leaves of mallow (*Malva sylvestris*) were collected from the farm at Etay-Elbarod city, El-Behera governorate, Egypt, season 2015. They were cleaned by water, air dried in oven at (40°C to 50°C) and grinded using an electric blender. The powder of sample was kept in polyethylene bags and preserved in deep freezer at (-18°C) until use.

All chemicals, solvents used in this study were purchased from El- Gomhoria Company of Chemicals and Drugs, Tanta City, Egypt.

### **2. Methods**

#### **2.1. Determination of proximate chemical composition**

Moisture content, crude protein content, Ether extract, Ash and crude fiber were determined as described in the A.O.A.C. (2005). All analyses were carried out in triplicates and the values were expressed as the mean  $\pm$  standard deviation ( $M \pm SD$ ) on dry weight basis. Total carbohydrates content was calculated by subtracting protein, ash and ether extract contents from the total mass of 100 and available carbohydrates were calculated by subtracting crude fiber content from total carbohydrates as reported by Tadrus (1989).

#### **2.2. Preparation of mallow extract**

Extract of mallow was prepared from ground dried green leaves using different

solvents (70% ethanol, 95% methanol and water) at ratio of 1 to 10 w/v the extraction was carried out in an orbital shaker at room temperature ( $22 \pm 2$  °C) for 24 hrs. After that, the extract was separated by filtration using Whatman No.1 filter paper. The filtered extract was kept in deep freezer at -18 °C until use Charles *et al.* (1993). To determine the extraction yield of samples, 10 ml of the extract was evaporated under vacuum in rotary evaporator at 45° C and weighted.

#### **2.3. Determination of total phenolic compounds content:**

Total phenolic content in the extracts were determined spectrophotometrically using folin-ciocalteau reagent according to the procedure of Thaipong *et al.* (2006). the stander curve was prepared using gallic acid.

#### **2.4. Determination of total flavonoids**

Total flavonoids were determined according to Vuong *et al.* (2014). The quercetin was used to prepare the stander curve.

#### **2.5. Identification and quantification of phenolics and flavonoids by HPLC**

Constituents of phenolics and flavonoids of mallow sample were identification and quantified using HPLC technique in National Research Center, Cairo, Egypt according to the method outlined by Evangelisti *et al.*, (1997).

#### **2.6. Determination of total tannins content.**

Tannins content of samples was estimated by using Folin-Danis method (Schanderi, 1970). The absorbance was measured by spectrophotometer (T80+UV/VIS) at 700 nm. The standard curve was produced by using of tannic acid and the results were expressed as

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mg of tannic acid equivalent per g of dry sample (mg TAE/g).

**2.7. Determination of total saponins:**

Total saponins content was estimated according to the method described by Hiai *et al.*, (1975) using 8% vanillin in ethanol and 72% sulphoric acid. The standard curve was produced by using of cholesterol and the results were expressed as mg of saponins equivalent per g of dry sample.

**2.8. Determination of antioxidant capacity by phosphomolybdenum method**

The antioxidant capacity of the *Malva sylvestris* extract was evaluated by the phosphor molybdenum method according to the procedure of Prieto *et al.*, (1999). The assay based on the reduction of molybdenum (VI) to molybdenum (V) by the antioxidant compounds and subsequent formation the green phosphate-molybdenum (V) complex, which has a maximal absorption at 695 nm at acidic pH. The antioxidant activity is expressed as mg equivalent of ascorbic acid at 500 ppm.

**2.9. Scavenging activity on DPPH free radical**

The ability of a compound to donate a hydrogen atom was assessed on the basis of the scavenging activity of the stable 2,2-diphenyl-2-picrylhydrazyl

(DPPH) radical that was determined according to the procedure of Lim and Quah (2007). The antioxidant activity was calculated according to the following equation at (100ppm, 200ppm, 300ppm and 500ppm):

$$\% \text{ radical scavenging activity} = [1 - (A_{\text{sample}} / A_{\text{control}})] \times 100$$

Where: A = is absorbance at 517 nm

**2.10. Statistical analysis**

Data are represented as the means ± standard error of mean (SE) for three replicates calculated by Microsoft excel. Analysis of variance (ANOVA) was applied for evaluating the significant of variances due to the used treatments of (p≤0.05) and the differences between means were further tested using the Dunc's and LSD squires multiple ranges. All the statistical methods were as outlined by SAS (2002-2003).

**RESULTS AND DISCUSSION**

**1. Chemical composition of mallow green parts**

As shown in Table (1), green parts of mallow leaves contain 74.2% moisture, 8.5% crude protein, 3.58% ether extract, 4.58% ash, 5.65% crude fiber and 77.69% available carbohydrates (on dry weight basis). These results indicate that green parts of mallow are rich in carbohydrates and ash content while they contain a moderate content of protein and low content of ether extract.

Table (1): Proximate chemical composition of mallow green parts (dry weight)

| Chemical composition    | Amount %      |
|-------------------------|---------------|
| Moisture                | 74.22 ± 0.301 |
| Ether extract           | 3.58± 0.120   |
| Crude protein           | 8.50 ± 0.1    |
| Crude fibre             | 5.65 ± 1.340  |
| Ash                     | 4.58±0.284    |
| Available carbohydrates | 77.69 ± 0.485 |
| Total carbohydrates     | 83.34± 0.347  |

Barros *et al.* (2010) found that mallow leaves contain 76.30% moisture, 12.15% protein, 2.76% fat, 13.53% ash and 71.46% carbohydrates (on dry weight basis). Tabaraki *et al.* (2012) found that moisture content of *Malva sylvestries* leaves ranged from 82.8 to 86.23%, protein content ranged from 2.4 to 3.2 %, ash content ranged from 13.1 to 14.8 %, crude fibre content ranged from 2.9 to 5% and fat content ranged from 0.16 to 0.3%.

## 2. Yield, total phenolics, total flavonoids, total tannins and saponins of mallow extracts

The results in Table (2) show that maximum extraction yield was obtained using water as solvent followed by that obtained using methanol but the lowest extraction yield was obtained using 70% ethanol. This result may be attributed to the substances which soluble in water such as polyphenolics, free amino acids, sugars, some vitamins and some minerals more than those soluble in alcohols.

The results in the same Table show that the amount of total phenolics and total flavonoids of mallow leaves extracted by water were significantly higher than those extracted by 70% ethanol and 95% methanol. The amount of total phenolics and flavonoids were

higher than those found by Conforti *et al.* (2008) who found that total phenolics and total flavonoids of dried mallow leaves were  $28 \pm 0.35$  mg ACE/g and 4.77 mg rutin/100g, respectively. Beghdad *et al.* (2014) determined the extraction yield, total phenolics and total flavonoids extracted from mallow leaves using 96% ethanol and found that these parameters were 26.14%, 24.12 mg gallic/g and 5.69 mg rutin/100g dry weight, respectively. The differences between the obtained results and these findings can be attributed to the different solvents used in the extraction process.

Total tannins content of 70% ethanolic mallow extract was the highest value (15.52 mg Tannic/g) followed by the value of water extract (8.64 mg Tannic/g) but the methanolic extract had the lowest one (4.99 mg Tannic/g) as shown in Table (2). The obtained results for tannins content were higher than that found by Tabaraki *et al.* (2012), who found the tannins content in mallow leaves extracted by 70% ethanol for 2 hrs ranged from 1.86 to 2.18 mg/g. In fact, the effects of tannins are mainly related to their interaction with proteins and tannin-protein complexes insoluble, consequently, protein digestibility is decrease.

Table (2). Yield, total phenolics, total flavonoids, total tannins and saponins of mallow extracts obtained using deferent solvents

| Component                          | Solvent type    |                |                 |
|------------------------------------|-----------------|----------------|-----------------|
|                                    | 70%ethanol      | 95%methanol    | Water           |
| Extraction yield%                  | 16.42 ± 0.17 c  | 18.84 ± 0.1 b  | 23.03 ± 0.1 a   |
| Total phenolics (mg gallic/ g)     | 57.30 ± 0.04 b  | 43.29 ± 0.53 c | 121.69 ± 1.34 a |
| Total flavonoids (mg quercetin /g) | 11.00 ± 0.01 b  | 11.03 ± 0.01 b | 14.01 ± 0.01 a  |
| Total tannins (mg tannic/ g)       | 15.52 ± 0.007 a | 4.99 ± 0.004 c | 8.64 ± 0.009 b  |
| Total saponins (mg cholesterol/g)  | 6.07 ± 0.008 a  | 4.56 ± 0.04 b  | ND*             |

Values are the means ± SD of three determinations. \*not determined

In each row, means with a similar letter are not statistically significant from each other.

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It could be noted that 70% ethanolic extract of mallow green parts contains 6.07 mg saponins/g dry sample while its methanolic extract contains 4.56 mg/g. Saponins are one of natural chemical compounds of many secondary plant metabolites which are abundance in various plant species (Hostettmann and Marston, 1995) and have diverse

biological properties (De Geyter *et al.*, 2007).

### **3. Identification and quantification of phenolic compounds of mallow extract**

The results of phenolic compounds composition in methanolic extract of mallow green parts (mg/kg dry sample) was recorded in Table (3).

Table (3): Composition of the phenolic compounds by HPLC analysis of mallow green parts extract

| Phenolic compounds     | As ppm  | As % of total |
|------------------------|---------|---------------|
| Pyrogallol             | 575.22  | 15.57         |
| Gallic                 | 50.03   | 1.35          |
| 4-Amino-benzoic        | 10.16   | 0.27          |
| Protocatechuic         | 110.37  | 2.99          |
| Catechin               | 546.43  | 14.79         |
| Catechol               | 19.21   | 0.52          |
| Chlorogenic            | 52.94   | 1.43          |
| Epicatchin             | 23.39   | 0.63          |
| P-OH-benzoic           | 89.86   | 2.43          |
| Caffeine               | 76.11   | 2.06          |
| Caffeic                | 47.15   | 1.28          |
| Vanillic               | 470.32  | 12.73         |
| P-coumaric             | 30.28   | 0.82          |
| Ferulic                | 141.41  | 3.83          |
| Iso-ferulic            | 16.52   | 0.45          |
| e-vanillic             | 1137.73 | 30.79         |
| Ellagic                | 58.6    | 1.59          |
| Alpha-coumaric         | 15.28   | 0.41          |
| Benzoic                | 118.24  | 3.20          |
| 3,4,5-methoxy-cinnamic | 7.51    | 0.20          |
| Coumarin               | 31.42   | 0.85          |
| Salicylic              | 62.8    | 1.70          |
| Cinnamic               | 3.93    | 0.11          |
| Total                  | 3694.91 | 100           |

Twenty three phenolic compounds were identified and quantified in mallow green parts extract (Table 3). It could be observed that e-vanillic is the major phenolic compound where it valued 30.79% of total phenolic compounds. Shelbaya *et al.* (2011) found that pyrogallol (1109.65 ppm) is the major phenolic compound in mallow leaves extract. Tabaraki *et al.* (2012) identified 18 compounds in mallow leaves extract and they found that 2-methoxy -4-vinylphenol was the major phenolic compound. The results in Table (3) indicate also that pyrogallol was the second major compound which amounted 15.57% followed by catechin (14.79%), vanillic acid (12.73%), ferulic (3.83%) and benzoic (3.20%). The other phenolics were present in low amounts, where each compound valued individually less than 3% of total phenolic compounds. The results show that coumaric, cinnamic and their derivatives of mallow extract were found in very low amounts.

#### 4. Identification and quantification of flavonoids of mallow extract

Identification of flavonoids compounds of mallow leaves methanolic extract maintained the presence of 21 flavonoids compound (Table 4) Luteo 6-arbinose 8-glucose is the major flavonoid compound in mallow leaves extract, where it valued 27.95% of total flavonoids followed by Kaemp.3,(2-p-comaroyl) glucose (19.58 %) and acacetin (13.32%). The results show also that hespiridin (6.98%), naringenin (6.23%) and naringin (4.88%) were found to be in moderate amounts. Rosmarinic and apigenin have the lowest amounts in mallow green parts extract. Billeter *et al.* (1991) identified the flavonoids of *M. sylvestris* leaves extract and found that gossypetin 3-sulphate-8-O-β-D-glucoside, hypolaetin 3'-sulphate, and three 8-hydroxyflavonoids were the major flavonoids constituents.

Table (4): Composition of the flavonoids by HPLC analysis of mallow green parts extract .

| Flavonoids                     | As ppm | As % of total |
|--------------------------------|--------|---------------|
| Luteo 6-arbinose 8-glucose     | 967.62 | 27.95         |
| Luteo 6-glucose 8arbinose      | 40.28  | 1.15          |
| Apig 6-rhamnose 8-glucose      | 106.53 | 3.07          |
| Naringin                       | 169.39 | 4.88          |
| Apig 6-glucose 8-rhamnose      | 22.71  | 0.65          |
| Hespiridin                     | 241.56 | 6.98          |
| Rutin                          | 69.11  | 2.00          |
| Quercetrin-3-o-glucoside       | 72.73  | 2.10          |
| Rosmarinic                     | 4.89   | 0.14          |
| Apig. 7-O-neohespiroside       | 54.01  | 1.55          |
| Apig. 7-glucose                | 41.06  | 1.18          |
| Kaemp. 3,7-dirhamoside         | 38.21  | 1.10          |
| Quercetrin                     | 69.51  | 2.01          |
| Quercetin                      | 20.69  | 0.60          |
| Kaemp.3,(2-p-comaroyl) glucose | 678.54 | 19.58         |
| Naringenin                     | 215.84 | 6.23          |
| Hespirtin                      | 80.86  | 2.33          |
| Kampferol                      | 34.41  | 0.98          |
| Rhamnetin                      | 59.85  | 1.72          |
| Apigenin                       | 12.44  | 0.36          |
| Acacetin                       | 461.33 | 13.32         |
| Total                          | 3461.6 | 100           |

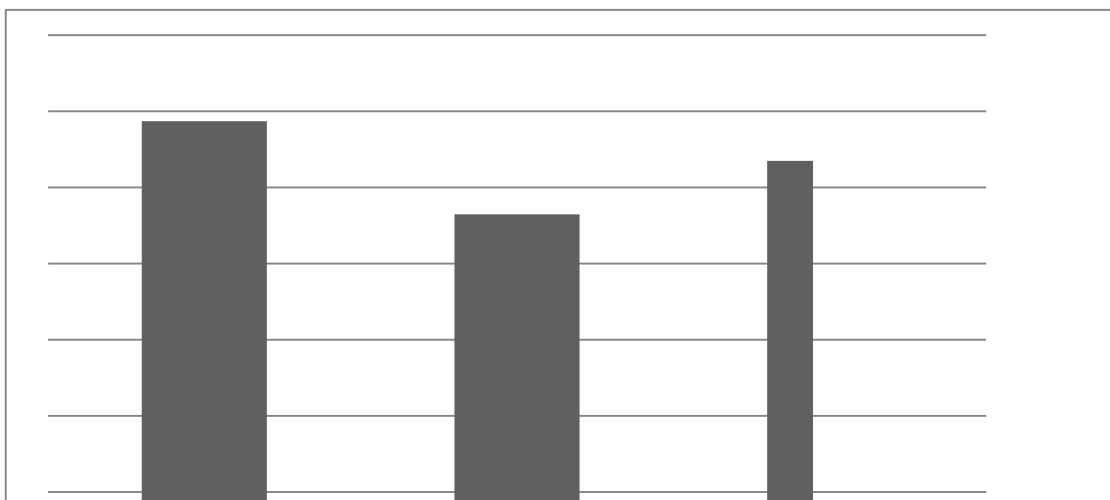
**5. Total Antioxidant Capacity (TAC) of mallow extract**

As shown in Fig. (1), total antioxidant capacity of 500ppm mallow green parts extract separated by 70% ethanol, 95% methanol and water decreased as the percentage of water in the solvent decreased. Whereas, the highest ( $p<0.05$ ) TAC was ( $58.7\pm0.1$  %) detected in the aqueous extract followed by 70% ethanolic extract ( $53.5 \pm 0.4$  %), while the 95% methanolic extract gave the lowest antioxidant capacity ( $46.5\pm0.4\%$ ). From the results in Table (2) and Fig (1), it could be said that the antioxidant capacity of mallow extract increased with increasing the total phenolics content.

**6. DPPH free radical scavenging activity**

Free radical scavenging is one of the known mechanisms of inhibition of lipid oxidation. In DPPH free radical scavenging assay, antiradical power of an antioxidant is measured as color changes from purple to yellow. It could be used to evaluate hydrogen-donating ability of the compound. The  $IC_{50}$  value (ppm) is the concentration at which the scavenging activity was 50% (Min-Sheng Su *et al.*, 2008). Also,  $IC_{50}$  values denote the concentration of sample, which is

required to scavenge 50% of DPPH free radicals (Ghasemi *et al.*, 2009). From the results in Table (5), it could be observed that antioxidant activity of the extracts obtained from dried green parts of mallow by different solvents was depending on the type of solvent and concentration of extracts. Where, the inhibition increased gradually with the increasing in concentration of extract. The results show that the highest values of inhibition were found to be in water extract followed by 70% ethanolic extract meanwhile the lowest values were detected in 95% methanolic extract at different concentrations. These results indicate that there is a positive relationship between total phenolics content in the extract and its antioxidant activity. The highest value of  $IC_{50}$  (370 ppm) was obtained in 95% methanolic extract followed by 70% ethanolic extract which has 360 ppm and the lowest value (280ppm) was detected in water extract. So, the higher concentration of extract exhibits the higher  $IC_{50}$  value. These results are in agreement with those mentioned by Beghdad *et al.* (2014), but they are lower than those reported by Conforti *et al.* (2008) and Ferreira *et al.* (2006).



**Fig. (1): Total antioxidant capacity (TAC%) of dried green parts of mallow extracts relating to antioxidant activity of ascorbic acid at 500ppm**

Table (5): DPPH free radical scavenging activity (as % inhibition) of green parts mallow extracts (ppm).

| Mallow leaves extracts | Inhibition (%) of DPPH in ppm |                       |                       |                       | IC <sub>50</sub><br>(ppm) |
|------------------------|-------------------------------|-----------------------|-----------------------|-----------------------|---------------------------|
|                        | 100 (ppm)                     | 200 (ppm)             | 300 (ppm)             | 500 (ppm)             |                           |
| 70% ethanol            | 29.9±0.11 <sup>b</sup>        | 35.8±0.5 <sup>b</sup> | 48.9±0.5 <sup>b</sup> | 67.4±0.4 <sup>b</sup> | 360                       |
| 95% methanol           | 26.7±1.2 <sup>c</sup>         | 32.2±0.5 <sup>c</sup> | 47.7±0.5 <sup>b</sup> | 65.8±1.2 <sup>b</sup> | 370                       |
| Water                  | 35.7±0.04 <sup>a</sup>        | 46.8±0.5 <sup>a</sup> | 51.6±0.2 <sup>a</sup> | 77.7±0.7 <sup>a</sup> | 280                       |

Means ± Standard deviation for three trails

In a column, means having the same superscript letters are not significantly different at 3% level

## Conclusion

Green parts of mallow contain considerable amounts of carbohydrates, protein and ash. Mallow leaves are a rich source for the bioactive compounds such as polyphenolics, flavonoids and saponins. Total antioxidant capacity (TAC) of mallow extracts increased as the percentage of phenolics content increased. Antioxidant activity of the extracts obtained from dried mallow using different solvents was depending on the type of solvent and concentration of extract. Hence, the mallow water extract at 500ppm had significantly higher antioxidant activity compared to other solvents. Consequently, mallow extracts could be used as natural antioxidants in some types of foods.

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## تأثير أنواع المذيبات علي المركبات الحيويه النشطه المستخرجه من اوراق الخبيزه

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### الملخص العربي

تأثير الاستخلاص بالمذيبات المختلفه (كحول ايثانول ٧٠% و كحول ميثانول ٩٥% والمستخلص المائي) علي المركبات النشطه بيولوجيا (البيوفينولات وفلافونويدات والسبونينات والتانينات) وقد أظهرت النتائج أن المركبات الفينولية الكلية المستخلصة من الأجزاء الخضريه للخبيزة باستخدام الإيثانول ٧٠% والميثانول والماء هي ٥٧.٢ و ٤٣.٢ و ١٢١.٦ ملجم / جم علي التوالي. محتوى الفلافونويدات الكلية من المستخلص المائي كان أعلى بكثير من تلك في المستخلص الميثانولي والإيثانولي. محتوى التانينات في المستخلص الإيثانولي ٧٠% (١٥.٥٢ ملجم تانيك / جم) كان أعلى بكثير بالمقارنة بالمستخلصات الأخرى وأقل كمية (٤.٩٩ ملجم تانيك / جم) كانت في المستخلص الميثانولي. كما تم الحصول علي نفس النتيجة بالنسبة للصابونين، حيث أن أعلى قيمة (٦.٧ ملجم / جم) كانت في المستخلص الإيثانولي. القدرة المضادة للأكسدة لمستخلص الخبيزة تزداد بزيادة تركيز المستخلص ولكنها تتخفف بزيادة تركيز الكحول في المذيب المستخدم في الإستخلاص. حيث أن أعلى قدرة للنشاط المضاد للأكسدة (٥٨.٧%) كان في المستخلص المائي وانخفضت تدريجيا بزيادة نسبة الكحول في المذيب. وأظهرت النتائج أيضا أن النشاط المضاد للأكسدة في مستخلصات الخبيزة يعتمد علي نوع المذيب المستخدم في الإستخلاص وتركيز المستخلص. فنجد أن المستخلص المائي عند تركيز ٥٠٠ ppm كان أعلى بكثير من النشاط المضادة للأكسدة للمستخلص الميثانولي بتركيز ١٠٠ ppm. ولذلك يعتبر الماء هو أحسن المذيبات لإستخلاص المركبات النشطة حيويا في الخبيزة. ونتيجة للنشاط المضاد للأكسدة الذي أظهره مستخلص الخبيزة يمكن استخدامه كمضاد أكسدة طبيعي في بعض أنواع الأطعمة.

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