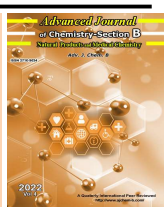
Advanced Journal of Chemistry-Section B
Natural Products and Medical Chemistry

Journal homepage: http://www.ajchem-b.com/



Original Research Article

Comparative Study of Several Extraction Solvents on Phenolic Profile, Toxicity and Antioxidants Potential of *Ephedra alata* Leaves

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ARTICLE INFO

Article history
Submitted: 2022-10-20
Revised: 2022-11-15
Accepted: 2022-11-30
Available online: 2022-12-15
Manuscript ID: AJCB-2210-1128
DOI: 10.22034/ajcb.2022.366558.1128

KEYWORDS
Ephedra Alata
Antioxidant Capacity
Total Polyphenols

ABSTRACT

Ephedra alata is one of the natural plants grow in AL-Salman Desert in AL-Muthana Governorate, Iraq and limited used in folk medicine. The most significance phytochemical constituents in plants are compounds considered antioxidants such as polyphenols. In general, the extraction conditions involving the technique employed and solvent type, greatly affect the anti-oxidant contents of herbal plants. The study aimed to evaluate the characteristics of some solvents like distilled water and other organic solvents such as ethanol, methanol, and acetone as the extraction solvents on efficiency, extraction yield, and antioxidant activity as well as determine the total polyphenol content (TPC), total flavonoids, and total tannins content of *Ephedra alata* leaves extracts. Overall the four different extracts obtained, the ethanol solvent exhibited the highest yield extraction, whereas methanol extract showed both higher antioxidant activity and total phenol content (TPC). Acetone showed lower antioxidant capacity and TPC compared with the three extracts. The obtained results are demonstrated that an increased phenolic content in *Ephedra alata* leaves extracts contributes to increase their antioxidant efficiency. Based on toxicity assessments, LD_{50} was measured (1150 mg/kg BW), this suggests the plant's safety.

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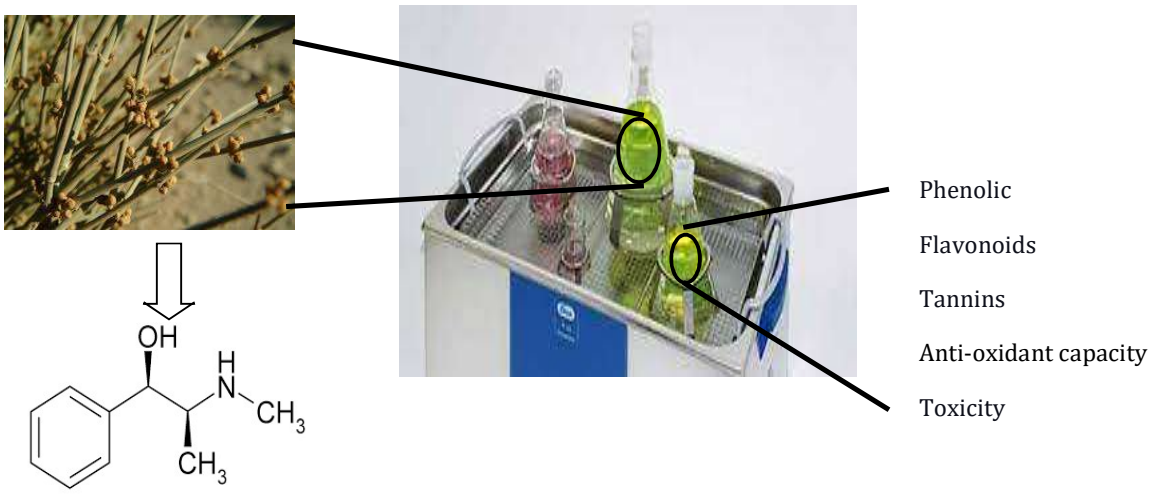
GRAPHICAL ABSTRACT



1. Introduction

Recently, a great interest has been attracted many researchers to focus on antioxidants and antimicrobial of herbal plants [1]. Plants are good sources of many bioactive compounds for a treat and maintaining human health [2]. Herbal plants therapy is considered useful for caring against many human diseases since oxidative stress as free radicals and reactive nitrogen or oxygen species responsible for generating damage cells diseases [1]. Antioxidants are group of substances used to control or inhibit oxidants due to their ability to defend against pathogenesis and injury tissues [3]. Herbs are rich of natural antioxidant potentials, such as phenolic compounds and other secondary metabolites [4]. Moreover, their ability as antioxidants, phenolic compounds in plants are proven to be as anti-microbial, anti-mutagenic, and anti-inflammatory properties. Extraction is a method to obtain metabolites in plants such as alkaloids, phenolics, flavonoids, glycosides, and others using selective solvents [5]. The solvent selectivity to extract the target compound from a plant material is related to the polarity [6]. Various organic solvents were used successfully to isolate and purify these effective antioxidants with high yields [7]. This study focus on determine toxicity, antioxidant potential, and phenolic compounds derived from one of the remedy plants (*Ephedra alata*) which grows in Al-Salman desert in Al-Muthanna governorate, south of Iraq using many extraction solvents [8]. The findings of this study discovered that methanol extracts from the plant selected have a high concentrations of antioxidants. To the best of our knowledge, this study is the first in Iraq sighting to evaluate the phytochemical components, antioxidants contents present in the natural plant *Ephedra alata*. However, further work is needed to identify the specific compounds that possess antioxidant properties in Ephedra, thus can be developed further on its applications for pharmaceutical uses.

2. Chemicals and Reagents

Aluminum chloride ($AlCl_3$), sodium hydroxide (NaOH), Folin-Ciocalteu (F-C) reagent, ascorbic acid, 95% ethanol, methanol 96%, acetone 98%, and DPPH (2, 2-Diphenyl picrylhydrazyl) reagent were purchased from Sigma Aldrich. Tannic acid, Sodium nitrite (≥ 99.0 % purity), Gallic acid, and Catechin reagents were from Sigma Aldrich. Sodium carbonates were

purchesed from Fluka Biochemika (Switzerland), quercetin, concentrated hydrochloric acid (37%) was purchased from Fluka.

2.1. Plant Material

The parts of the herbal plant *Ephedra alata* (Figure 1) were obtained and collected from the Al-Salman Desert in Al-Muthanna governorate, Iraq 200 km (124 miles) south of Samawah (Figure 2), from May to September (2020), the leaves were dried, powdered, stored in amber bottles, and kept in a dark dry place for extraction and analysis.

Figure 1. *Ephedra alata* in Salman in Muthanna, Iraq

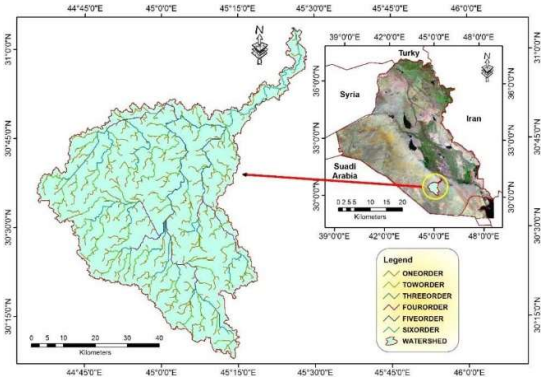


Figure 2. Location of area study.

2.2. Preparation of Extracts

The dried *Ephedra* parts (leaves) extracts were obtained by sonication (Elmasonic S 150). In separated experiments, 2.5 gm of powdered dry leaves with 50 mL of solvent (water, ethanol, methanol, and acetone) for 40 min at (40 ± 3 °C). The different extracts were stirred for 10 min (200 rpm). The supernatant of each extract was transferred to another tube and centrifuged at room temperature for 10 min at 2000 rpm. Evaporated and stored at 4 °C until analysis [9].

2.3. Total Phenol Content

The total phenolic content of plant leaves extract of *Ephedra alata* was examined by Folin-Ciocalteu colorimetric method based on oxidation-reduction reaction as reported by [10]. Gallic acid is used as the standard reference since it is less costly and essentially available than the other alternatives. The absorbance measurements were carried out on a Gallic acid solution using a UV-VIS spectrophotometer at wavelength of 760 nm. Three replications experiments were performed and the results are expressed as mg of Gallic acid per g of dry sample.

2.4. Total Flavonoid Concentration

Total flavonoids were determined based on aluminum chloride colorimetric assay reported by [11]. The absorbance of the extract solution was measured with spectrophotometer at a wavelength of 434 nm and the TFC results were expressed in mg which is equivalent to quercetin per gram of *Ephedra* extract.

2.5. Total Tannins

Tannins contents of extracts were quantified by spectrophotometric analysis according to the Folin Ciocalteuas described by [12]. Tannic acid was used as a standard. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE /g of the dry extract [13].

2.6. DPPH Assay

The method to measure free radical property according to the assay described by [14]. The extracts with the concentrations (5, 10, 50, and 100 μg mL^{-1}) were added to 0.5 mL of methanol

solution of DPPH. The absorbance was estimated versus a blank at 517 nm after incubation for 54 min at room temperature. The percentage scavenger of free radical DPPH was calculated using the following formula:

Scavenging effect (%) = (control absorbance - sample absorbance)/control absorbance x 100.

3. Results and Discussion

3.1. Extraction Yield

The extraction yield of *Ephedra alata* in different solvents varied from 8.22±0.27 to 18.72±0.91% (Figure 3). The ethanol was the best extraction solvent resulted in the highest level of the total bioactive compounds from the selected herbal plant. On the other hand, the use of acetone as an extraction solvent yielded small quantities of phenolic compounds when compared with the other solvents. The variations described the difference in polarities of the fourth solvents; a wide range of compounds with a high polarity can be isolated by solvents with high polarity [8].

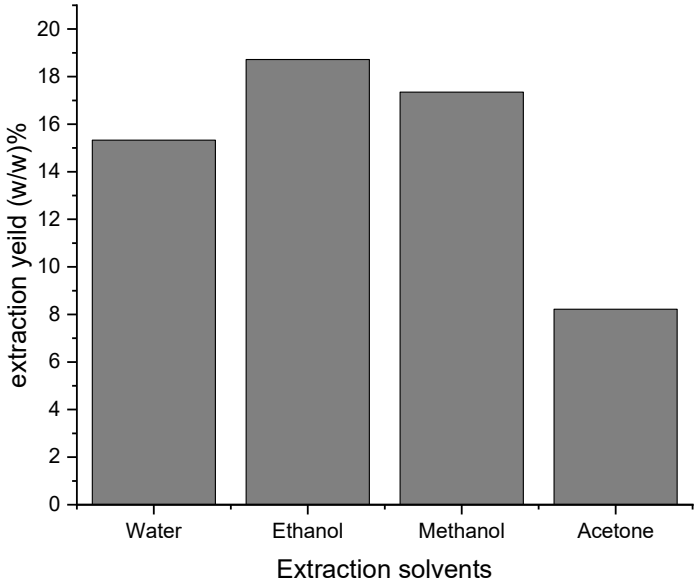


Figure 3. *Ephedra alata* leaves extraction yield (% DM) of the different solvents.

3.2. Total phenols, flavonoids, and tannins

Total phenolics, tannins, and flavonoids measured in the selected plant. The total phenolics and tannins content of *Ephedra alata* herbal extracts were estimated using Folin-Ciocalteuas reagent [15], while flavonoids content was determined by aluminum chloride methods [16]. These results obtained for extraction bioactive compounds from *Ephedra alata* using water, ethanol methanol, and acetone extracts of the plants are presented in Table 1.

Table 1. The total polyphenol, flavonoid, and tannin concentrations in different extracts

Solvent	Polyphenols mg/g	Flavoniods mg/g	Tannins mg/g
Water	1.8 ± 0.00	3.5 ± 0.01	3.8 ± 0.03
Ethanol	2.4 ± 0.02	4.8 ± 0.06	4.1 ± 0.01
methanol	4.6 ± 0.07	4.9 ± 0.01	5.8 ± 0.00
acetone	3.9 ± 0.62	4.6 ± 0.04	4.3 ± 0.01

3.3. Antioxidant activities

The antioxidant properties of different solvents extracts of *ephedra alata* medicinal herb were evaluated by DPPH free radical scavenging [17] (Figure 4).

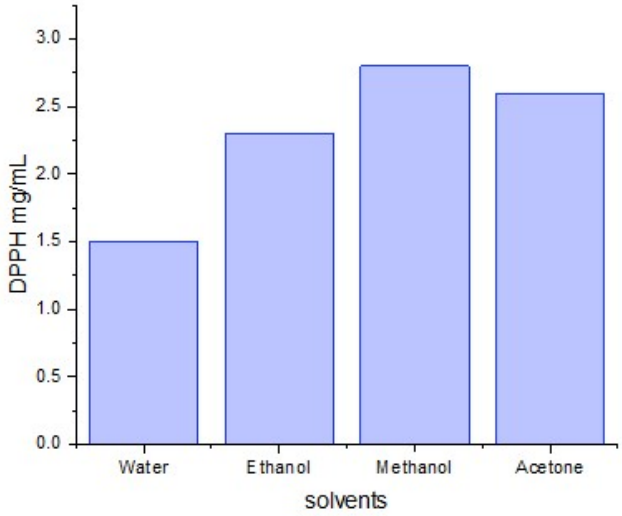


Figure 4. Antioxidant activities of different solvents extracts of *Ephedra alata.*

3.4. Acute Toxicity Assessments (Animals and treatment)

Fasted adult rats were divided into five groups of eight animals per group. Aqueous *Ephedra alata* extract was orally administrated to the experimental animals. The groups treated orally dosed with *Ephedra alata* (100, 200, 400, 500,700, 900, 1000, 1300, 1700, and 2300 mg/kg BW) of the Ephedra extract, respectively. There were no signs of toxicity or body weight changes up to 14 days for the selected. No death occurred in animals up to 2300 mg/kg of aqueous *Ephedra alata* extract. The acute oral LD50 value of *Ephedra alata* was calculated as 1150 mg/kg body weight. Thus, it could be concluded that the *Ephedra alata* observed no toxic signs till the dose range of 2300 mg/kg B.W, as illustrated in Figure 5.

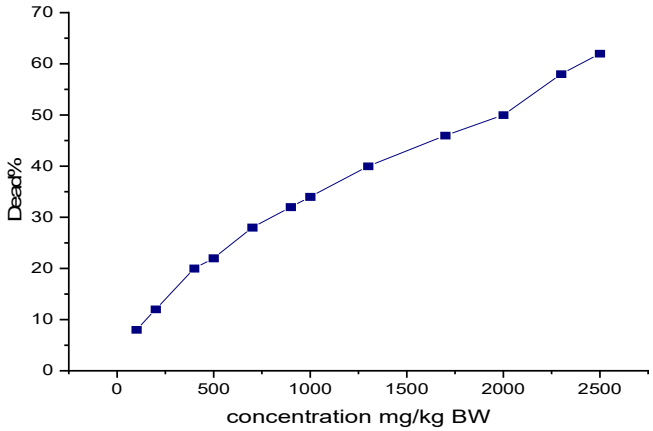


Figure 5. LD_{50} dose response curve of *Ephedra alata*

3.5. Effect of Different Extraction Solvents on Total Phenol Content of *Ephedra Alata* Extracts

Phenolic compounds are considered as the most phytochemical metabolites generated by plants to protect them from oxidative stress damage [18]. These important natural phytochemicals act as free radicals scavengers [19]. They also play a vital role as natural antioxidants by donating electrons to stabilize, prevent free radical action and damage [20]. Moreover, the biological antioxidant activity of herbal plant extract is due to the presence of phenolic compounds. Moreover, considerable attention has been elevated greatly in efficiency these important biological compounds isolated from herbal plants to utilize them as a natural antioxidant for health benefits [21]. The efficiency of polyphenolic extraction depends on the proper extraction solvent used in extraction [22]. In the present research, the extraction of *Ephedra alata* leaves to obtain the highest content of TPC using four solvents different in their polarities. Table 1 presents TPC, TFC, and total tannins of *Ephedra alata* leaves extracts from common solvents (water, methanol, ethanol, and acetone). The highest total phenolic levels produced from the methanolic extract (4.6 ± 0.07 mg GAE/g *Ephedra alata* extract), whereas acetone extract (3.9 ± 0.62 mg GAE/g *Ephedra alata* extract). Furthermore, Table 1 lists the total flavonoids levels and tannins levels of *Ephedra alata* leaves extracts. These results indicated that the methanol extract seems to be more effective and suitable for polyphenols extraction from *Ephedra alata* leaves.

3.6. Effect of Different Extraction Solvents on Antioxidant Powerful of *Ephedra alata* leaves Extract

In recent years, many reports focused on the antioxidant properties levels affected by the different polarity solvents of the obtained extracts [23-26]. All the results of extracts tested to the DPPH radical method revealed that the greatest free radical scavenging potential of *Ephedra alata* leaves was found in methanol extract. This can be illustrated by the great solubility of the secondary bioactive compounds due to the methanol polarity. DPPH is the most common antioxidant compound to determine the antioxidant activity of plant extracts in a short time [27]. The DPPH radical scavenging of different *Ephedra alata* extracts using ascorbic acid as a standard were depicted in Figure 4. The extracts obtained from acetone showed the higher significant radical scavenging activity compared with the other solvents. Moreover, the solvent ethanol indicated the remarkably antioxidant potential of *Ephedra alata* extracts. Moreover, according to the obtained current results, the increased scavenging ability of extracts is directly in agreement with higher contents of TPC. In the case of determining the antioxidant power and total phenolic values of the different extracts, this present study indicates that the highest level of total phenol content and antioxidant properties can be obtained when methanol is used as solvent extraction.

3.7. Acute Toxicity

The present study revealed safety of *Ephedra alata* extract. It was found that the aqueous herbal extract was found to be safe and produced no death or signs of toxicity up to 2300 mg/kg BW. To investigate the effect of the *Ephedra alata* on mortality and toxic potential, different level doses of the aqueous extract were tested attributable to the oral use of this extract caused neither little change in the general behavior nor toxicity at the dose (100 to 2300 mg/kg). However, it was observed a dose increase in symptoms of toxicity and death rate. These findings could be indicated safety evaluation of *Ephedra* extract, as demonstrated in Figure 5.

4. Conclusions

The present study indicated the evaluation of several extraction solvents for their capacity to extract antioxidant and phenolic contents from *Ephedra alata* leaves. Among the tested extraction solvents, methanol exhibited as the suitable solvent for the highest yield of phytochemical compounds amounts extracted from *Ephedra alata* including phenolics, flavonoids, and tannins. Based on the obtained results, it can be concluded that the methanolic extract of *Ephedra alata* provided the highest antioxidant properties among the used solvents. These findings suggest that methanol was a highly efficient solvent to obtain the highest value of bioactive compounds which are responsible for the desired pharmaceutically materials. The higher LD50 measured is generally considered the herbal plant safe. However, further tests were required to discover more efficacy compounds for their beneficial effects on human health.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

References:

[1] J J. Sharifi-Rad, M.Hoseini-Alfatemi, M. J. A. Teixeira da Silva, Antibacterial, antioxidant, antifungal and anti-inflammatory activities of crude extract from Nitraria schoberi fruits. *Biotech*., 5 (2015) 677–684.

[2] J. Sharifi-Rad, S.M. Hoseini-Alfatemi, A. Miri, Phytochemical screening and antibacterial activity of Prosopis farcta different parts extracts against methicillin-resistant Staphylococcus aureus (MRSA). *Minerva Biotecnol*., 26 (2014) 287–293.

[3] A. Das Sarma, R.A. Mallick, K.A. Ghosh,. Free radicals and their role in different clinical conditions: an overview. *Int. J. Pharm. Sci. Res*., 1 (2010) 185–192.

[4] S. Sen, R. Chakraborty,. The role of antioxidants in human health. *ACS Symposium Series 1083, American Chemical Society, Washington DC*, (2011) 1–37.

[5] M.G. Miguel, Antioxidant activity of medicinal and aromatic plants. A review. *Flavour. Fragr. J*., 25 (2010) 291–312.

[6] B.J. Salas, R.T. Tellez, P.J.M. Alonso, V.F.M. Pardo, M. Capdevila, G. Rodriguez, Chemical composition and antioxidant activity of the essential oil of Thymbra capitata (L.) Cav, *Acta Bot. Gallica*., 157 (2010) 55–63.

[7] H. Djeradi, A. Rahmouni, A. Cheriti. Antioxidant activity of flavonoids: a QSAR modeling using Fukui indices descriptors. *J. Mol. Model*., 20 (2014) 2476.

[8] WC. Evans: Elsevier Health Sciences;. Trease & Evans' Pharmacognosy. , 16th ed. London 2009.

[9] Environmental Protection Agency 3550B,. Ultrasonic Extraction. Revision 2. Washington, (1996)1-5.

[10] D. Harvey, Modern Analytical Chemistry. First Edition, Mc Graw-Hill.,(2000).

[11] H.I. Ismail, K.W. Chan, A.A. Mariod, M. Ismail, Phenolic content and antioxidant activity of cantaloupe (Cucumis melo) methanolic extracts. *Food Chem*, 119 (2000) 643–647.

[12] S. tannin- Bakari, M. Ncir, S. Felhi, H. Hajlaoui, M. Saoudi, N. Gharsallah, A. Kadri, Chemical composition and in vitro evaluation of total phenolic, flavonoid, and antioxydant properties of essential oil and solvent extract from the aerial parts of Teucrium polium grown in Tunisia. *Food Sci. Biotech*., 24 (2015) 1943-1949.

[13] R.B. Broadhurst, W.T. Jones,. Analysis of condensed tannins using acidified vanillin, *J. Sci. Food Agr*., 48 (1978) 788–794.

[14] T. Hatano, H. Kagawa, T. Yasuhara, T. Okuda, Two new flavonoidsand other constituents in licorice root their relative astringencyand radical scavenging effect. *Chem. Pharm. Bull*., 36 (1988) 2090–2097.

[15] P. Siddhuraju, K. Becker Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringa oleifera Lam.) *leaves J. Agric. Food Chem*., 51 (2003) 2144-2155.

[16] R.G. Woisky, A. Salatino, Analysis of propolis: Some parameters and procedures for chemical quality control*. J. Apicult. Res*., 37 (2015) 99–105.

[17] M.S. Blois, Antioxidant determinations by the use of a stable free radical. *Nature*, 181 (1958) 1199–1200.

[18] L. Yang, K. S.Wen, X. Ruan, Y. X. Zhao, F. Wei, and Q. Wang,"Response of plant secondary metabolites to environmental factors,"*Molecules*, 23 (2018) 762.

[19] O. K. Chun, D. Kim, C. Y. Lee, "Superoxide radical scavenging activity of the major polyphenols in fresh plums," *J Agric. Food Chem*, 51 (2003) 8067–8072.

[20] P. Vardakas, Z. Skaperda, F. Tekos, A.F. Trompeta, A. Tsatsakis, C.A. Charitidis, D. Kouretas, An integrated approach for assessing the in vitro and in vivo redox-related effects of nanomaterials. *Environ Res.,* 197 (2021) 11-20.

[21] M. Leopoldini, T. Marino, N. Russo, and M. Toscano, "Antioxidant properties of phenolic compounds: H-atom versus electron transfer mechanism," *J. Phys.Chem A*, 108 (2004) 4916–4922.

[22] C. Caleja, A. Ribeiro, M.F. Barreiro, I.C. Ferreira, "Phenolic compounds as nutraceuticals or functional food ingredients," *Curr. Pharm. Des*, 23 (2017) 2787–2806.

[23] H. Zhao, J. Dong, J. Lu et al., "Effects of extraction solvent mixtures on antioxidant activity evaluation and their extraction capacity and selectivity for free phenolic compounds in barley(Hordeum vulgare L.)" *J Agric. Food Chem* 54 (2006) 7277–7286.

[24] AK Garg, M Faheem, S Singh, Role of medicinal plant in human health disease. *Asian J Plant Sci Res*., 11 (2021) 19-21.

[25] F. Tekos, S. Makri, Z.V. Skaperda, A. Patouna, K. Terizi, I.D. Kyriazis, Y. Kotseridis, E.V. Mikropoulou, G. Papaefstathiou, M. Halabalaki, D. Kouretas, Assessment of antioxidant and antimutagenic properties of red and white wine extracts in vitro. *Metabolites*., 11 (2021) 436-439.

[26] A. Mokrani, K. Madani, Effect of solvent, time and temperatureon the extraction of phenolic compounds and antioxidant capacity of peach (Prunus persica L.) fruit, *Sep. Purif. Technol.* 162 (2016) 68–76.

[27] S. B. Kedare, R. P. Singh, "Genesis and development of DPPH method of antioxidant assay," J F S T., 48 (2011) 412–422.

HOW TO CITE THIS ARTICLE

Muna Hasson Saoudi, Ihab Abbas Taher, Comparative Study of Several Extraction Solvents on Phenolic Profile, Toxicity and Antioxidants Potential of Ephedra alata Leaves, Ad. J. Chem. B, 4 (2022) 239-246.

DOI: 10.22034/ajcb.2022.366558.1128
URL: http://www.ajchem-b.com/article_162962.html