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Research Article
Phytochemical and Heavy Metal Composition of Various Extracts of *Ephedra sinica* from Jordan

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Abstract

Background and Objective: *Ephedra* is used in traditional medicine overall, the world. It has a suggestive efficient anti-diabetic, antibacterial, antioxidant anti-asthmatic and anticancer activity. The present study was designed to evaluate the phytochemical potential, secondary metabolites and heavy metals content of different extracts of *Ephedra sinica* grown in Jordan. **Materials and Methods:** Dry leaves of *E. sinica* were extracted sequentially with ethanol, methanol, chloroform, acetone, n-hexane, ethyl acetate and water. Extracts were filtered, evaporated and tested for phytochemical components of flavonoids, tannins and total alkaloids, besides, heavy metal analysis for copper, cobalt, lead, cadmium and nickel. **Results:** The highest extract yield was obtained from water extract (56%) whereas hexane showed the lowest yield (7.5%). Ethanol extract showed the highest contents of flavonoids and tannin contents of (100.7±1.5 mg QE g^{-1} DW) and (44.03±7.4 mg GAE g^{-1} DW), respectively. The crude powder extract of *E. sinica* showed the acceptable amount of alkaloids of 17.4±1.3 mg g^{-1} DW, with normal values of heavy metal concentration. **Conclusion:** The yield and phytochemical components from *E. sinica* leaves are solvent-based. Further research is suggested to be conducted for any pharmacological potential of *E. sinica* phytochemical components.

Key words: Alkaloids, *E. sinica*, flavonoids, heavy metal, phytochemical, tannin

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Jordan is naturally gifted with a wide range of plant species based on its diverse climate. A total number of 2,543 species, 868 genera and 142 families, was recorded. At least 20% of them showed medicinal potential and were reported in folk medicine, as well as in the pharmaceutical industry. Most of the recorded taxa are wild plants, in addition to a few species, which are cultivated[1,2]. One member of this group is *Ephedra sinica* or Ma huang. This species grows mostly in the Dana region, near Tafila, south Jordan[3]. *E. sinica* belongs to the Ephedraceae family. It is an evergreen shrub, has dark green stems and can be distinguished from other kinds of *Ephedra* species by the reddish-brownish base of leaf sheaths and a more delicate habit[3,4].

Ephedra is known as one of the oldest herbs used in traditional medicine in Jordan and overall, the world. It has a suggestive efficient anti-diabetic, antibacterial, antioxidant anti-asthmatic and anticancer activity. Some reports indicated its role in treating cold, cough, fever, allergy and cardiovascular diseases[3,5,6]. For years, several studies have been carried out to evaluate the phytochemistry and bioactivity of the genus Ephedra. A wide range of natural products was indicated, including; anthocyanin, tannins, saponins, phenolic acids, flavonoids and essential oils. Besides, alkaloids, ephedrine and pseudoephedrine, were found in some kinds of *Ephedra* species, their role in central nervous system stimulation, bronchodilation and vasoconstriction, has been recorded. Moreover, ephedrine toxicity was associated with stroke, myocardial infarction and sudden death[5-11].

Recent scientific research indicated a possible toxic, mutagenic, carcinogenic potential of some medicinal plants. Some reports recorded that food supplements containing E-type alkaloids represent an unacceptable health risk as a consequence of their misuse or abuse[12]. Their pharmacological and toxicological effects were related to the individual alkaloid type E enantiomeric structure and receptor binding characteristics[13]. Besides, Khaleel[14] reported that heavy metals are presented in many herbal formulations, showing a suggestive adverse and toxic effect on a human being; since having phytotoxic potentials in nature. Behera and Bhattacharya[15] recommended assessment of the medicinal plant's products before their use.

The Literature documented that the therapeutic effect of herbal medicine differs greatly due to the respective phytochemical constituents they contain and the use of different anatomical parts or their processing methods[16-20]. *E. sinica* has outstanding pharmaceutical importance however, to our best knowledge, the literature lacks data regarding its total contents and phytochemical composition. Therefore, in this study, an attempt to elucidate the identification and quantification of the phytochemical constituents as well as the heavy metals concentration in different leaf extracts of *E. sinica* from Jordan was done. The result of this study will add more value to *E. sinica* used to protect the human body against diseases.

MATERIALS AND METHODS

Study area: This research was conducted from March, 2019 to January, 2021.

Plant material and extraction: The aerial parts of *E. sinica* were picked from Tafila city, South Jordan. The plant was washed with deionized water, dried at room temperature, powdered with a mechanical grinder and stored in an air-tight container until further use. The powder was extracted successfully with ethanol, methanol, chloroform, acetone, n-hexane, ethyl acetate and water; with occasional shaking; for two days, at room temperature. The extracts were filtered through a membrane filter and concentrated in a rotary evaporator at 40°C and stored at -20°C for further analysis[21].

Quantitative phytochemical analysis

Determination of total flavonoid content: The aluminium chloride colorimetric method was used for flavonoids determination[22]. The crude of the different extract samples ((mg mL^{-1} methanol) or quercetin (standard)) was separately added to a volumetric flask containing; 1.5 mL of ethanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1M sodium acetate and 2.8 mL of distilled water. The extract solution was kept for 30 min in a dark place, at room temperature. The absorbance of the reaction mixture was measured at 415 nm by UV/V is spectrophotometer. The result was expressed as mg of quercetin equivalents per gram of extract dry weight (mg QE g^{-1} DW).

Determination of tannin content: The tannins were determined using a modified Folin-Ciocalteu method[23]. Each extract (0.1 mL) or gallic acid (standard) was added to a volumetric flask containing 7.5 mL of distilled water, 0.5 mL of Folin-Ciocalteu phenol reagent and 1 mL of 35% Na_2CO_3 solution. The mixture was diluted by adding distilled water up to 10 mL. The mixtures were shaken well and kept at room

temperature for 30 min. The absorbance was measured against the blank at 725 nm with a UV/Visible spectrophotometer. The tannin content was expressed regarding mg of gallic acid equivalent/g of extract dry weight (mg GAE g^{-1} DW).

Determination of the total alkaloid content: About 200 mL of 10% acetic acid dissolved in ethanol was added to 5 g of dry powdered sample, covered and allowed to stand for 4 hrs. Samples were filtered and concentrated on a water bath. The final volume of each sample was reduced up to 1/4th the original sample volume. Concentrated ammonium hydroxide was added dropwise until the precipitation was complete. Later, the precipitate was collected and washed with dilute NH_4OH and filtered. The remaining residue is the alkaloid was dried and weighed[24].

Heavy metals analysis: According to Buchmann *et al.*[25], 10 mL of 65% HNO_3 was added to 2 g of dried powdered sample and stayed overnight at room temperature. The digest was heated at 85°C to evaporate acid. Then, 1 mL of 30% H_2O_2 was added, filtered and diluted with deionized water to 50 mL. Standard solutions of copper (Cu), Cobalt (Co), lead (Pb), cadmium (Cd) and nickel (Ni) were prepared. The concentrations of heavy metals in the prepared *E. sinica* leaf extracts were measured by using Optical Emission Spectrophotometer (Optima 2000, PerkinElmer, USA).

Statistical analysis: The one-way analysis of variance (ANOVA) and Tukey's multiple comparisons at ($p<0.05$) were conducted using SPSS statistical software (version 10). Data were reported as Means±SD of three measurements.

RESULTS

The yield of extracts: The yield percentage of different *E. sinica* extracts are presented in Fig. 1. Yields range from 7.5-56%, to where the significant highest extractive yield ($p<0.05$) was obtained from water extract (56%), followed by methanol (18.1%) chloroform (16.5%), ethanol (15.7%), acetone (14.5%), ethyl acetate (12%) and n-hexane (7.5%) extract.

Total flavonoid content: The concentration of flavonoids in various plant extracts of *E. sinica* was determined using the spectrophotometric method with aluminium chloride. The content of flavonoids was expressed concerning quercetin equivalent the standard curve Eq.:

$$y = 0.015x+0.005$$

$$R^2 = 0.999$$

in Fig. 2. Ethanol extract exhibited significantly ($p<0.05$) the highest total flavonoid content (100.7±1.5 mg QE g^{-1} DW of extract), followed by n-hexane (83.0±7.5), ethyl acetate (71.40±1.2), methanol (68.1±0.1), acetone (37.0±4.5), chloroform (36.3±4.1) and water (6.12±0.1).

Determination of tannin contents: The tannin contents in the examined extracts of *Ephedra sinica* using the Folin-Ciocalteu's reagent was evaluated about gallic acid equivalent the standard curve Eq.:

$$y = 0.0.13x+0.23$$

$$R^2 = 0.981$$

The highest concentration of tannin was measured in ethanol extract (44.03±7.4 mg GAE g^{-1} DW), while the lowest concentration was obtained from chloroform extract (8.17±2.1 mg GAE g^{-1} DW) in Fig. 3.

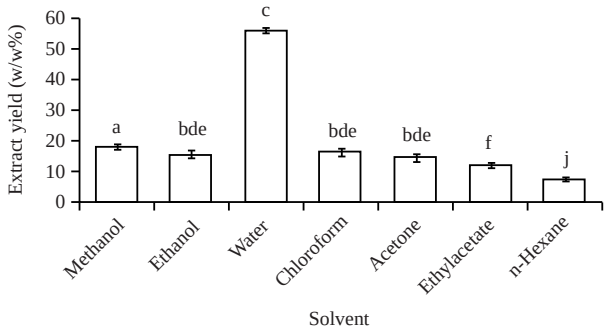

Fig. 1: Extract yield percentage of different extracts of *E. sinica*
Value is the mean average of three replications for each Solvent±Standard deviation, columns not sharing the same superscript letter are significantly different ($p<0.05$)

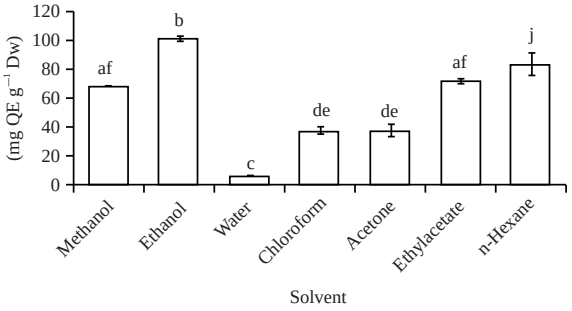

Fig. 2: Total flavonoid content of different extracts of *E. sinica* expressed as mg of quercetin equivalents per gram of dry extract
Value is the mean average of three replications for each Solvent±Standard deviation

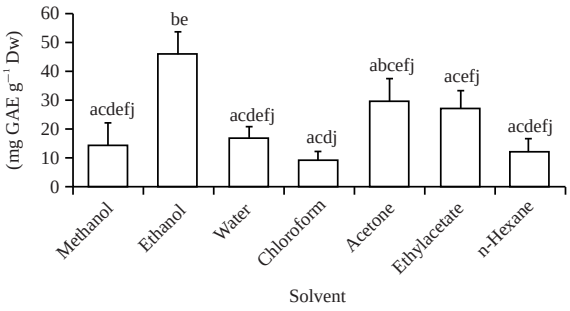

Fig. 3: Total tannin content of different extracts of *E. sinica* expressed as gallic acid equivalent/g of dry extract
Value is the mean average of three replications for each Solvent±Standard deviation, columns not sharing the same superscript letter are significantly different (p<0.05)

Table 1: The concentration of heavy metals in dry aerial parts of *E. sinica*

Elements	Concentration (ppt)
Nickel (Ni)	0.325
Cobalt (Co)	(Under the limit of the instrument)
Lead (Pb)	0.475
Cadmium (Cd)	0.1
Copper (Cu)	9.825

Total alkaloid content: The alkaloid content was found to be 17.4±1.3 mg g^{-1} DW.

Heavy metals analysis: Results have been presented in Table 1. Elemental analysis showed copper content was highest in the samples of crude extract of *E. sinica*. Other elements determined including the heavy metals were present in trace amounts. Cobalt concentration was too small to be detected using the available analytical technique.

DISCUSSION

The phytochemical analysis is one of the methods used to explore bioactive compounds in plants. In the present study, results of the quantitative analysis of the phytochemicals show the presence of a high quantity of flavonoids, tannins and alkaloids, in various extracts derived from the leaves of *Ephedra sinica.* All of these phytochemicals, which are widely distributed across the plant kingdom are found to possess high medicinal activities. Among many plant chemicals, phenolic compounds, especially flavonoids and tannins are the most important for dietary applications, besides they have been the most extensively researched[26]. They have gained tremendous interest in their therapeutic agents against a wide variety of diseases, most of which correlated to oxidant damage[27,28]. Alkaloids are very important in medicine and have been indicated as precursors for the synthesis of useful drugs.

In the present study, the extraction yield by various solvents decreased in the following order: 56% water >18.1% methanol >16.5% chloroform >15.7% ethanol >14.5% acetone >12% ethyl acetate >7.5% n-hexane. The highest yield percentage was obtained in water extract (Fig. 1). This may be attributable to the higher solubility of proteins and carbohydrates in water and methanol compared to ethanol and acetone[29]. Many reports documented that increasing the water concentration in the solvent enhances the extraction yield of some medicinal plants[30,31]. However, higher extraction yield does not necessarily imply having a high concentration of the bioactive compounds[32].

The results of this study showed that the used solvents had a significant effect on the extraction efficiency of phytochemicals from leaves of *Ephedra sinica* (<0.05). Among all solvents used in this study, the extract obtained by ethanol showed the highest values of flavonoids (100.7±1.5 mg QE g^{-1} DW) and tannin (44.03±7.4 mg GAE g^{-1} DW). The lowest concentration of flavonoids was measured in water extract, while the chloroform extract exhibited the lowest concentration of tannin (Fig. 2 and 3). Previous studies revealed that the extraction solvent plays an important role in the extraction of important secondary bioactive compounds from medicinal plants. Studies indicated that the presence of various antioxidant compounds with different biological and chemical characteristics and polarities may or may not be soluble in a particular solvent[31,33]. Ethanol has been known as a good solvent for polyphenol extraction and is safe for human consumption[34]. A similar result was reported by Al-Rimawi *et al.*[35,] who analyzed the total flavonoid contents of *E. alata* Decne using three different solvents namely, water, 100% EtOH and 80% EtOH. The results showed that total flavonoids content was higher in the case of 100% EtOH (19.5±0.3 mg QE g^{-1} DW). However, *E. sinica* extract in the present study contains more flavonoid constituents.

The total alkaloid content was measured in the powder leaves of *Ephedra sinica* and was found to be 17.4±1.3 mg g^{-1}. According to the literature survey of Ephedra species, it was found that not all of these species contain alkaloids[36]. However, lower quantities of alkaloids were obtained from other species such as *E. distacyha* subsp. *Helvetica* (15.8±2.0 mg g^{-1} dry weight) and *E. major* using the spectrophotometric method[7]. The total amount of alkaloids isolated from *Ephedra alata* aerial parts was 0.2-0.22%.

Although heavy metals are naturally present in the environment, their deposit in the plant induce stress and cause various diseases. Copper is one of the heaviest elements

essential for the normal physiological functioning of living organisms but at a high concentration, can be harmful. Besides, lead is another heavy metal with an adverse poisonous effect; it can accumulate in the tissues over the years. It is on the list of the most dangerous metals, since it can cause acute and chronic environmental contamination[37,38]. The analytical determinations of metals in medicinal plants are a quality control required to certify their purity, safety and efficacy. Reports revealed that the distribution of the heavy metals among plant organs is selective and based on the plant part to be studied, plant organs` surface characteristics and the heavy element to be examined[39]. According to the earlier published work by WHO cited in many reports, the specific limit of the heavy metals: Cu, Pb, Ni, Cd and Co, in medicinal plants and food are 10, 10, 1.5, 1 and 0.2 ppm, respectively[15,40-42]. The concentration of heavy metals in an investigated crude powder of the *Ephedra sinica* leaves were found to be less than standard limits. In addition, cobalt concentration was found to be less than the detection limit, hence does not possess any harm to the products obtained (Table 1).

Previous pharmacological studies revealed that *Ephedra* species possess several biological activities such as antioxidant, antimicrobial, antidiabetic, hepato-protective and cardiovascular effects. Since biologically active compounds occur naturally in very small concentrations, the choice of a definite extraction method and the corresponding suitable solvent is an important step in the drug discovery process[17,18,31]. This study, therefore, could provide some biochemical basis for the ethnomedical use of extracts from *E. sinica* in the treatment of many diseases.

CONCLUSION

The quantitative phytochemical analysis of the *E. sinica* leaves extracts reveals the presence of a high quantity of flavonoids, tannins and alkaloids, in various extracts. The most suitable solvent is ethanol. On the other hand, heavy metal elements are present within the permissible limits. Further studies are required to isolate and characterize the active agents of *E. sinica*.

SIGNIFICANCE STATEMENT

This study discovered the phytochemical potential of several extracts of *E. sinica* grown in Jordan. The high yield of flavonoids, tannins and alkaloids can be beneficial for pharmacological studies. This study will help the researchers to uncover the biochemical basis of new drug discovery for some diseases. Further research is suggested to isolate the active gradients to be used to develop new pharmaceutical drugs, as an alternative to routinely used ones and to be a good choice with an expected cheap and safe herbal fashion.

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REFERENCES

1. Afif, F.U. and B. Abu-Irmaileh, 2000. Herbal medicine in Jordan with emphasis on special less commonly used medicinal herbs. J. Ethnopharmacol., 72: 101-110.
2. Abu-Irmaileh, B.E. and F.U. Afifi, 2003. Herbal medicine in Jordan with special emphasis on commonly used herbs. J. Ethnopharmacol., 89: 193-197.
3. Abu-Shandi, K., H. Al-Soufi, M. Al-Gababsheh and J. Al-Saudi, 2017. Identification of the phytochemical composition of two species of *Ephedra* plants-traditional anticancer herbs-by gas chromatography-mass spectrometry. J. Chem. Pharm. Res., 9: 5-10.
4. Kakiuchi, N., M. Mikage, S. Ickert-Bond, M. Maier-Stolte and H. Freitag, 2011. A molecular phylogenetic study of the *Ephedra distachya*/*E. sinica* complex in Eurasia. Willdenowia, 41: 203-215.
5. Lee, M., 2011. The history of *Ephedra* (ma-huang). J. Royal Coll. Physicians Edinburgh, 41: 78-84.
6. Thakur, A. and S.R. Pathak, 2018. Introduction to medicinally important constituent from chinese medicinal plants. In: synthesis of medicinal agents from plants. Tewari, A. and S. Tiwari (Eds.,). Elsevier Ltd. Amsterdam, Netherlands, pp: 333-349.
7. Ibragic, S. and E. Sofic, 2015. Chemical composition of various *Ephedra* species. Bosnian J. Basic Med. Sci., 15: 21-27.
8. Al-Snafi and A. Esmail, 2017. Therapeutic importance of *Ephedra alata* and *Ephedra foliata* a review. Indo Am. J. Pharm. Sci., Vol. 4.
9. Cruz, A., I.I. Padilla-Martínez and M.E. Bautista-Ramirez, 2018. Ephedrines as chiral auxiliaries in enantioselective alkylation reactions of acyl ephedrine amides and esters: A review. Curr. Org. Synth., 15: 38-83.
10. Mighri, H., A. Akrout, N. Bennour, H. Eljeni, T. Zammouri and M. Neffati, 2019. LC/MS method development for the determination of the phenolic compounds of tunisian *Ephedra alata* hydro-methanolic extract and its fractions and evaluation of their antioxidant activities. South Afr. J. Bot., 124: 102-110.

11. Elhadef, K., S. Smaoui, M. Fourati, H.B. Hlima and A.C. Mtibaa *et al*., 2020. A review on worldwide *Ephedra* history and story: From fossils to natural products mass spectroscopy characterization and biopharmacotherapy potential. Evidence-Based Compl. Alt. Med., Vol. 2020. 10.1155/2020/1540638.
12. Miller, S.C., 2004. Safety concerns regarding ephedrine-type alkaloid-containing dietary supplements. Mil. Med., 169: 87-93.
13. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) 2013. Scientific opinion on safety evaluation of ephedra species for use in food. EFSA J., Vol. 11. 10.2903/j.efsa.2013.3467.
14. Khaleel, S.M.J., 2018. Studying the heavy metals composition and the impact of different common solvents on the extraction efficiency of phytochemical secondary metabolites from the leaves of *Ziziphus spina-christi* grown in Jordan Pak. J. Nutr., 17: 392-398.
15. Behera, B. and S. Bhattacharya, 2016. The importance of assessing heavy metals in medicinal herbs: A quantitative study. TANG Humanitus Med., 6: 3-3.
16. Bhandary, S.K., N.S. Kumari, V.S. Bhat, K.P. Sharmila and M.P. Bekal, 2012. Preliminary phytochemical screening of various extracts of *Punica granatum* peel, whole fruit and seeds. J. Health Allied Sci. NU, 02: 34-38.
17. Chigayo, K., P.E.L. Mojapelo, S. Mnyakeni-Moleele and J.M. Misihairabgwi, 2016. Phytochemical and antioxidant properties of different solvent extracts of *Kirkia wilmsii* tubers. Asian Pac. J. Trop. Biomed., 6: 1037-1043.
18. Ngo, T.V., C.J. Scarlett, M.C. Bowyer, P.D. Ngo and Q.V. Vuong, 2017. Impact of different extraction solvents on bioactive compounds and antioxidant capacity from the root of *Salacia chinensis* L. J. Food Qual., Vol. 2017. 10.1155/2017/9305047.
19. Khaleel, S.M.J., A.S. Jaran and M.Y.S. Haddadin, 2016. Evaluation of total phenolic content and antioxidant activity of three leaf extracts of *Ziziphus spina-christi* (Sedr) grown in Jordan. Br. J. Med. Med. Res., 14: 1-8.
20. Khaleel, S.M.J., 2018. Anti-α-Glucosidase, anti-α-amylase and anti-inflammatory effects of leaf extracts of *Ziziphus spina-christi* (Sedr) grown in jordan. Res. J. Biol. Sci., 13: 1-7.
21. Khaleel, S.M.J. and M.Y.S. Haddadin, 2013. The enhancement of hawthorn leaf extracts on the growth and production of short chain fatty acids of two probiotic bacteria. Pak. J. Nutr., 12: 144-149.
22. Guo, D.J., H.L. Cheng, S.W. Chan and P.H.F. Yu, 2008. Antioxidative activities and the total phenolic contents of tonic Chinese medicinal herbs. Inflammopharmacology, 16: 201-207.
23. Tambe, V.D. and R.S. Bhambar, 2014. Estimation of total phenol, tannin, alkaloid and flavonoid in *Hibiscus tiliaceus* Linn. wood extracts. Res. Rev.: J. Pharmacogn. Phytochem., 2: 41-47.
24. Harborne, J.B., 1973. Phytochemical methods: A guide to modern techniques of plant analysis. Springer, Dordrecht, London, ISBN: 978-94-009-5921-7, pp: 1-32.
25. Buchmann, J.H., J.E.D. Sarkis and C. Rodrigues, 2000. Determination of metals in plant samples by using a sector field inductively coupled plasma mass spectrometer. Sci. Total Environ., 263: 221-229.
26. Balasundram, N., K. Sundram and S. Samman, 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence and potential uses. Food Chem., 99: 191-203.
27. Ross, J.A. and C.M. Kasum, 2002. Dietary flavonoids: Bioavailability, metabolic effects and safety. Annu. Rev. Nutr., 22: 19-34.
28. Khaleel S.M., A.S. Jaran and T. Muhammad-Fateh Al-Deeb, 2019. Antimicrobial and lipid peroxidation inhibition potential of ziziphus spina-christi (sedr), a jordanian medicinal plant. Journal of Biological Sciences 19: 131-136.
29. Zielinski, H. and H. Kozlowska, 2000. Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions. J. Agric. Food Chem., 48: 2008-2016.
30. Chatha, S.A.S., F. Anwar, M. Manzoor and J. Bajwa, 2006. Evaluation of the antioxidant activity of rice bran extracts using different antioxidant assays. Grasas y Aceites, Vol. 57. 10.3989/gya.2006.v57.i3.56.
31. Sultana, B., F. Anwar and M. Ashraf, 2009. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules, 14: 2167-2180.
32. Singh, A., V. Bajpai, M. Srivastava, K.R. Arya and B. Kumar, 2014. Rapid profiling and structural characterization of bioactive compounds and their distribution in different parts of *Berberis petiolaris* Wall. Ex G. don applying hyphenated mass spectrometric techniques. Rapid Commun. Mass Spectrom., 28: 2089-2100.
33. Do, Q.D., A.E. Angkawijaya, P.L. Tran-Nguyen, L.H. Huynh, F.E. Soetaredjo, S. Ismadji and Y.H. Ju, 2014. Effect of extraction solvent on total phenol content, total flavonoid content and antioxidant activity of *Limnophila aromatica*. J. Food Drug Anal., 22: 296-302.
34. Dai, J. and R.J. Mumper, 2010. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules, 15: 7313-7352.
35. Al-Rimawi, F., S. Abu-Lafi, J. Abbadi, A.A.A. Alamarneh, R.A. Sawahreh and I. Odeh, 2017. Analysis of phenolic and flavonoids of wild *Ephedra alata* plant extracts by LC/PDA and LC/MS and their antioxidant activity. Afr. J. Trad., Compl. Alt. Med., 14: 130-141.
36. Zhang, B.M., Z.B. Wang, P. Xin, Q.H. Wang, H. Bu and H.X. Kuang, 2018. Phytochemistry and pharmacology of genus *Ephedra*. Chin. J. Nat. Med., 16: 811-828.
37. Toplska, K., K. Sawicka-Kapusta and E. Cieslik, 2004. The effect of contamination of the krakow region on heavy metals content in the organs of bank voles (Clethrionomys glareolus, Schreber, 1780). Pol. J. Environ., 13: 103-109.

38. Wani, A.L., A. Ara and J.A. Usmani, 2015. Lead toxicity: A review. Interdiscip. Toxicol., 8: 55-64.
39. Blagojević, N., B. Damjanović-Vratnica, V. Vukašinović-Pešić and D. Đurović, 2009. Heavy metals content in leaves and extracts of wild-growing *Salvia officinalis* from montenegro. Polish J. Environ. Stud., 18: 167-173.
40. Miller-Cebert, R.L., N.A. Sistani and E. Cebert, 2009. Comparative mineral composition among canola cultivars and other cruciferous leafy greens. J. Food Compos. Anal., 22: 112-116.
41. Yap, C.K., M.R.M. Fitri, Y. Mazyhar and S.G. Tan, 2010. Effects of metal-contaminated soils on the accumulation of heavy metals in different parts of *Centella asiatica*: A laboratory study. Sains Malaysiana, 39: 347-352.
42. Bhat, R., K. Kiran, A.B. Arun and A.A. Karim, 2010. Determination of mineral composition and heavy metal content of some nutraceutically valued plant products. Food Anal. Methods, 3: 181-187.