Global Journal of Pharmacology 7 (4): 412-415, 2013
ISSN 1992-0075

DOI: 10.5829/idosi.gjp.2013.7.4.8149

Heavy Metal Analysis of Seabuckthorn Leaf Extract

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Abstract: Seabuckthorn [SBT, *Hippophae rhamnoides* L.] is a very popular plant of high altitude cold desert areas having tremendous medicinal properties and well reviewed in Chinese and Tibetan literature. 75% ethanolic extract of SBT leaves has already been reported to possess many pharmacological activities but there are no reports on heavy metal analysis of this extract. The present study was undertaken to analyze the heavy metal content of this extract with respect to Big Four heavy metals As, Pb, Hg and Cd using Inductively Coupled Plasma-Mass Spectrometry [ICP-MS]. The findings of this study was compared with prescribed limits of these metals in WHO guidelines and the content of all these heavy metals were found to be within safe limits. These findings indicate that 75% ethanolic SBT leaf extract is safe from the point of view of heavy metal toxicity and being a good source of antioxidants this extract have the possibilities to be used in combination therapy along with a good chelating agents for reversal of heavy metal toxicities.

Key words: 75% Ethanolic Extract · Heavy Metal Toxicity · Inductively Coupled Plasma-Mass Spectrometry

INTRODUCTION

There are three integral aspects of any drug, namely quality, safety and efficacy. All these three aspects are interdependent. However, it can be said that quality of a drug is established based on the factors which influence its safety and efficacy. One of the major hindrances in the global recognition of herbal drugs is lack of standard quality control profile. Quality control of raw material is one of the major issues for defining quality profile of finished product [1]. In case of herbal drugs, optimizing the quality of raw material i.e. plant material is a big challenge since they are of natural origin. As plants are produced from soil and besides that also, they need various facilities like optimum sun light, irrigation and additional nutrients in form of various metallic elements etc for proper growth and development. On consideration of these points, it appears that there are many factors which may influence the quality of plant material being used for drug development. Hence, it is very much required to evaluate the quality of plant materials before using it for drug development.

Heavy metal analysis is one of the parameters which have been established for evaluating the quality of plant materials [2]. Elements can be divided into two categories. First category consists of highly toxic elements like Pb, Cd, Hg and As while second category includes elements which may have been introduced during processing such as Cr, Cu, Ni etc. The first category elements [also called as "Big Four"] must be measured in all the samples. These elements are of potential toxic nature and they must be kept within their safe limits [3]. Heavy metals are chemical elements with a specific gravity that is at least 5 times the specific gravity of water. The specific gravity of water is 1 at 4°C (39°F). Some well-known toxic metallic elements with a specific gravity that is 5 or more times that of water are arsenic, 5.7; cadmium, 8.65; lead, 11.34; and mercury, 13.546 [4].

Hippophae rhamnoides L., commonly known as Seabuckthorn [SBT], is a high altitude plant, found in cold desert areas [5]. SBT is a good source of a large number of nutrients, phytochemicals and bioactive substances [6,7]. The medicinal effects of SBT have been suggested to be due to the presence of high antioxidant contents [6, 8]. Almost all parts of this plant are having medicinal property, leaf being one of them. In the previous studies [9], 75% ethanolic extract of SBT leaves has been found to be one of the best in bioactivity guided extraction and

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also to have beneficial effects in scopolamine induced cognitive impairment in rats [10]. All this investigations indicate towards the need for heavy metal analysis of this extract.

MATERIAL AND METHODS

Collection of Plant Material: Seabuckthorn (*Hippophae rhamnoides*) leaves were collected from Leh, India in the month of September and authenticated by National Institute of Science Communication and Information Resources (NISCAIR). During and after harvesting, Seabuckthorn leaves were transported and stored in non-metallic containers. Leaves were cleaned and washed thoroughly with demineralized water in order to prevent any metal contamination. Washed leaves were dried under shade in a clean, dust free environment and used for extract preparation.

Preparation of Extracts: Ethanolic extract (75%) of Seabuckthorn leaves were prepared by cold percolation method. The powdered Seabuckthorn dry leaves were soaked with 75% ethanol (Raw material to solvent ratio 1:8) at room temperature for 24 hrs and filtered with 80 mesh nylon cloth. This process was repeated 5 times for complete extraction. The filtrates obtained after each extraction were combined and stored at ambient temperature. The combined filtrates were again filtered with 250 mesh nylon cloth to get the liquid extract. This extract was then concentrated under reduced pressure using Buchi Rotavapor till a solid mass was obtained.

Digestion of Plant Samples and Analysis of Heavy Metals: In brief, 0.5 gm of the extract was transferred to a MRS vial and 5 ml of concentrated HNO_3 was added and digested by using MRS. The sample was digested until the clear solution was obtained. The digested solution was cooled and made to the final volume of 100 ml with Mili-Q water. Sample solutions were then filtered through membrane (0.45micron) filter. Finally, the digested samples were used for metal analysis using Inductively Coupled Plasma Mass Spectrometry (Perkin Elmer DRC-e Model) [11]. Each sample was digested in triplicate.

RESULTS AND DISCUSSION

Heavy metal toxicity is one of the major impediments in the global acceptance of herbal drugs. The development of herbal drugs involved various processing steps like cultivation, collection, harvesting etc, which may itself act as a source of heavy metal contamination.

Table 1: Heavy metal analysis of 75% ethanolic SBT leaf extract

Element	Concentration (mg/kg)	Upper limit (mg/kg)
Arsenic (As)	0.424 ± 0.02	5.00
Lead (Pb)	1.765 ± 0.10	10
Mercury (Hg)	0.304 ± 0.01	0.50
Cadmium (Cd)	0.007 ± 0.01	0.30

SBT is a tremendous source of various nutrients with a wide range of pharmacological activities. In bioactivity guided extraction of SBT leaves, 75% ethanolic extract has been found to be one of the best extracts with reference to antioxidant activity and total phenol content [9]. As discussed earlier, 75% ethanolic SBT extract is rich in polyphenolic compounds and these compounds are known for their chelating properties [12]. On the other hand, almost all the heavy metals studied in the present investigation have a tendency of bioaccumulation. So it can be hypothesized that polyphenolic compounds present in the extract may cause chelation of heavy metals present in the extract and after ingestion these compounds may get metabolized inside the body leaving the heavy metals inside, in a way these polyphenolic compounds would act as a transporter for heavy metals. Hence, there are more possibilities of heavy metal toxicity on intake of this SBT extract. In light of these aspects, the quantitative evaluation of heavy metals in 75% ethanolic SBT leaf extract becomes quiet significant.

WHO guidelines [2] suggest the upper limit for As in herbal products as 5 mg/kg. In the present investigation, the concentration of As was found to be 0.424 mg/kg which indicates that 75% ethanolic SBT leaf extract is safe with respect to arsenic content. Pb is a non degradable toxic element with tendency of getting accumulated inside the body. It is potentially harmful to both children and adults [13]. As per the results (Table 1), the concentration of Pb in SBT extract was found to be 1.765 mg/kg which is under the safe range of prescribed limits [2], it indicates that 75% ethanolic extracts of SBT leaves are safe with respect to Pb toxicity. Further, Hg is a non essential heavy metal. Nervous system is very sensitive to all forms of Hg. It may cause permanent brain damage. Again, SBT extract was found to be safe as far as Hg toxicity was concerned. Similarly, in case of Cd also, 75% ethanolic SBT leaf extract was quiet safe.

On comparison with available literature on heavy metal analysis of SBT extract, findings of the present investigation were found to be similar to that of study conducted by Saggu *et al.* [12]. However, these authors did not analyze 75% ethanolic extract instead they analyzed aqueous extracts of SBT fruits and leaves.

Moreover, toxic effects of heavy metals have been suggested to be due to oxidant and antioxidant imbalance towards oxidants (oxidative stress) and also due to high affinity of these metals for thiol groups of functional proteins. It has also been suggested that a combination therapy using a chelating agent along with an antioxidant can be used as an effective approach for reversal of toxic effects caused by heavy metals [14, 15]. Now, since 75% ethanolic extract of SBT leaves has already been reported to have good antioxidant properties and results (Table 1) indicate that it is safe with respect to all the big four toxic heavy metals, it can be hypothesize that intake of this extract may cause beneficial effects in case of heavy metal toxicity incidents. However, further studies are required to confirm it.

Further, it is very well known that SBT is a good source of various antioxidants and nutrients. Even, various therapeutic effects of SBT extracts have also been proposed to be due to presence of wide range of antioxidants [5-7]. Being a good source of polyphenolics and rich in antioxidants, 75% ethanolic SBT leaf extract is likely to have a potential to be developed as a nutraceutical/ dietary supplement and results of the present investigation also support it. Attrey *et al.*, [10] have suggested the positive influence of this extract in lipid peroxidation which signifies its antioxidant effect in oxidative stress conditions. This, once again indicate the possibility of positive influence which is likely to be produced on administration of this extract in the conditions of heavy metal toxicity.

CONCLUSION

The concentration of all the analyzed heavy metals in 75% ethanolic extract of dried Seabuckthorn leaves were within the acceptable limits. Thus, it can be concluded that this extract is safe for further product development.

ACKNOWLEDGEMENT

The authors are grateful to the Chief Controller R and D (Life Sciences) and Director, Life Sciences, DRDO HQ, DRDO Bhavan, New Delhi for providing funds for the project. The authors are also grateful to Dr. Ashok K. Chauhan, Hon'ble Founder President, Amity University Uttar Pradesh, Noida, for providing facilities for conducting the research.

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