



Preliminary Phytochemical Screening and Heavy Metal Analysis of Leaf Extracts of *Hippophae Rhamnoides* Linn

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ABSTRACT

The present study was aimed to investigate the preliminary phytochemical screening of the leaves of *Hippophae rhamnoides* L belonging to family *Elaeagnaceae*. The dried leaves of the plant were subjected to successive Soxhlet extraction by continuous hot percolation method using organic solvents of increasing polarity such as chloroform, n-hexane, petroleum ether, ethyl acetate and ethanol. The aqueous extract was prepared by cold maceration technique. All the extracts were subjected to qualitative phytochemical screening and it showed the presence of active constituents such as alkaloid, flavonoids, phenol and tri-terpenoids. Heavy metals and inorganic elements are determined by inductively coupled plasma optical emission spectrometry technique. The results obtained shows that the extracts contain medicinally important bioactive constituents and also heavy metals present in the plant extracts were within the permissible limits. This justifies its use in the traditional medicine for the treatment of different diseases such as asthma, heart disorders, chest pain, high cholesterol and antioxidant.

Keywords: Heavy metal and inorganic element analysis, *Hippophae rhamnoides*, Phytochemical screening.

INTRODUCTION

Seabuckthorn (*Hippophae rhamnoides* L., *Elaeagnaceae*), growing at high altitude of 2500-4000 m, is a dwarf to tall (2-5m) plant. The plant contain wide range of bioactive substances and used in traditional medicines. The plant grows in the regions of Himachal Pradesh, Uttarakhand, Ladakh and Sikkim and Arunachal Pradesh in north-east India.¹ In the year 1962, Indian scientists reported the anti-tumour effect of seabuckthorn bark.^{2,3} The oil and juice, commercially produced from its fruits and extracts from leaves, are used in Russian traditional and official medicines for the wound healing, antibacterial, as anti-ulcer and anti-inflammatory (antiphlogistic) agent and as the multivitamin product.⁴



Figure 1: Illustrate the botanical view of *Hippophae rhamnoides* L

In Russia, the plant extracts are commonly used as the components of various medicinal, compositions in dermatology, stomatology, ophthalmology, veterinary and cosmetology etc. A chemical composition and biological action of active principles from different organs of *Hippophae rhamnoides* have been reviewed in a number of monographs.⁵ In China, positive effects of seabuckthorn oil on heart diseases have also been

reported.⁶ Seabuckthorn fruit oil has also been found to possess curative effect on atopic dermatitis.⁷ *Seabuckthorn* oil also has wound healing effects.⁸

Several Studies have been carried out on the bio-chemical characteristics revealed that fruit, leaves and other parts of seabuckthorn are very rich sources of variety of anti-oxidants like carotenoids, tocopherols, flavonoids and other bioactive substances with medicinal properties^{9,10}, therefore it has a strong anti-oxidant and immunomodulatory properties¹¹ etc., which make this plant a very promising raw material for the preparation of products for health protection and treatment of various diseases. The present paper describes about the Preliminary Phytochemical Screening and Heavy Metal Analysis of Leaf extracts of *Hippophae rhamnoides* L.

MATERIALS AND METHODS

The leaves of the *Hippophae rhamnoides* were collected from Madhgaon, a low altitude place in semi-arid region of Lahaul, Shogo, a high altitude place in arid region of Spiti, from Tinu village of Lahaul. The leaves of *Hippophae rhamnoides* were dried at 65°C in an oven for approximately 5-7 days till the dry weight will be constant manually crushed and stored at 4°C until further use (max. 3-6 months). All experiments will be performed with the same dried plant seed material.

Preparation of the Extracts

The shade dried, coarsely powdered leaf material was extracted successively with chloroform, n-hexane, petroleum ether, ethyl acetate and ethanol using Soxhlet apparatus by continuous hot percolation method. The aqueous extract was prepared by cold maceration

technique. Then the extracts were collected, concentrated using rotary vacuum evaporator.

Qualitative Phytochemical Analysis

Freshly prepared extracts were subjected to phytochemical evaluation for the detection of various constituents using conventional protocol.¹²

Test for terpenoids

(a) *Salkowski test*: To the Petroleum ether extract, 10ml of CHCl_3 and 5ml of conc. H_2SO_4 was carefully added. A reddish brown layer formation at the interface showed the presence of terpenoids. (b) *Lieberman Burchard test*: The test was performed on a TLC plate using chloroform: methanol (10:1) as the mobile phase. The plates were sprayed with Lieberman Burchard reagent and viewed under UV at 366nm. Green fluorescence confirmed the presence of terpenoids.

(b) *Lieberman Burchard reagent*: 1 ml of conc. H_2SO_4 , 20 ml of acetic anhydride and 50 ml of chloroform.

Test for Saponins

The ethanolic and aqueous extracts were diluted separately with distilled water for the formation of foam which indicated the presence of saponins.

Test for Phenols and Tannins

About 0.5g of the crude aqueous and ethanolic extracts were dissolved in 5 ml of distilled water, filtered and mixed with ferric chloride reagent. Formation of a blue-green precipitate showed the presence of phenolic and tannin compounds.

Test for flavonoids

(a) To 5 ml of dilute ammonia solution a portion of the aqueous filtrate of the plant extracts were added which was followed by the addition of concentrated H_2SO_4 . A yellow coloration indicated the presence of flavonoids. (b) Few drops of 1% aluminum solution were added to a portion of each filtrate. A yellow coloration indicated the presence of flavanoids.

Test for alkaloids

To 5 ml of chloroform, alcoholic and water extracts, 3ml of diluted hydrochloric acid was added and the solutions were filtered. The filtrates were tested with different alkaloid reagents like Mayer's and Wagner's reagent. A cream and reddish brown precipitate confirmed the presence of alkaloids.

Preparation of Mayer's reagent: About 1.36 gm of Mercuric chloride was dissolved in 60 ml distilled water (a). And 5 gm of Potassium iodide in 20 ml of distilled water (b). The solution (a) and (b) were adjusting the volume to 100 ml with distilled water.

Preparation of Wagner's reagent: Dissolve 1.27 gm of iodine and 2 gm of potassium iodide in 5 ml of water and make up the volume to 100 ml distilled water.

Test for carbohydrates & glycosides

The alcoholic and aqueous extracts were separately taken in 5 ml of test tube containing distilled water and then filtered. The filtrate was subjected to Molish's test. The formation of a violet ring with both the crude plant extracts confirmed the presence of carbohydrates and glycosides.

Test for phytosterols

The petroleum ether and ethyl acetate extracts were refluxed with potassium hydroxide for about 10 minutes. The extracts were then diluted with distilled water and were re-extracted with diethyl ether and finally subjected for Liebermann's burchard test. Both the extracts showed a deep green color precipitate which indicated the presence of phytosterols.

Liebermann's Reagent: A mixture of 5ml of acetic anhydride with 2ml of conc. H_2SO_4 .

Test for steroids

Two ml of acetic anhydride was added to 0.5g ethanolic extract of each sample with 2ml of H_2SO_4 . The color changed from violet to blue (or) green in some samples indicating the presence of steroids.

Test for fixed oils and fats

a) *Spot test*: 5mL of n-hexane plant crude extract solution placed in an ordinary paper. A translucent spot is visible. This indicates the presence of fat.

Qualitative analysis & Quantitative estimation of Heavy metal and Inorganic elements

- Qualitative determination of Heavy metal and Inorganic elements was done by the standard methods.
- Quantitative estimation of Heavy metal and Inorganic elements is determined by inductively coupled plasma optical emission spectrometry method.

RESULTS AND DISCUSSION

Extractive values

The extractive values of chloroform, n-hexane, petroleum ether, ethyl acetate, ethanol and water extracts are given in the Table 1.

Table 1: Represents the extractive values of different crude plant extracts

Type of Extract	Yield (% w/w)
Chloroform	2.19
n-hexane	3.76
Petroleum Ether	3.09
Ethyl Acetate	7.49
Ethanol	11.20
Aqueous	4.27

Table 2: The results of qualitative phytochemical analysis

Plant Constituent	n-hexane	Pet. ether	CHCl ₃	Ethyl acetate	Ethanol	Water
Alkaloids	----	----	++++	----	++++	++++
Carbohydrates & glycosides	----	----	----	----	++++	++++
Phytosterols	----	++++	----	----	++++	----
Phenols & Tannins	----	----	----	----	++	++++
Terpenoids	----	++++	++++	----	----	----
Saponins	----	----	----	----	++++	++++
Flavanoids	----	----	----	----	----	++++
Fixed oil and fats	++++	----	----	----	----	----
Steroids	----	----	----	++++	----	----

Qualitative Phytochemical Analysis

The results of qualitative phytochemical analysis are given in the Table 2. It revealed the presence of carbohydrate, amino acids, alkaloids, flavonoids, phenolic compounds and terpenoids present in the extracts.

Heavy metals and Inorganic elements

The growth of medicinal plants not only need nutrients for normal plant growth, but also can selectively uptake and accumulate some trace elements which are good and may also be toxic for human health if there not within the limits.

Table 3: Heavy metal content of leaves of *Hippophae rhamnoides*

Element	Results (ppm/ml)	Specification
Lead	0.042	> 10ppm
Cadmium	0.019	>0.3ppm
Arsenic	0.051	>5ppm
Mercury	0.0011	>0.5ppm
Chromium	0.027	>10ppm

Table 4: Inorganic elements of Leaves of *Hippophae rhamnoides*

Element	Results (ppm/ml)
Aluminum	35.78
Copper	0.192
Calcium	0.22
Iron	4.703
Manganese	0.043
Potassium	0.192
Sodium	0.217
Manganese	0.094
Zinc	0.679
Nickel	0.441

The results obtained shows that *Hippophae rhamnoides* contains inorganic element of Al, Cu, Ca, I, Mn, K, Na, Mg, Zn, Ni were found to be 35.78, 0.192, 0.22, 4.703, 0.043,

0.192, 0.217, 0.094, 0.679, 0.441µg/ml and heavy metal such as Arsenic (As), Cadmium (Cd), Lead (Pb), Mercury (Hg) were found to be 0.015, 0.009, 0.043, 0.0012 respectively.

CONCLUSION

The preliminary phytochemical analysis of *Hippophae rhamnoides* reveals the presence of alkaloid, flavonoids, phenolic content and terpenoids which could attribute to the medicinal efficacy. Heavy metal and inorganic elements are present within the permissible limits. Furthermore studies are required to isolate and characterize the active principles of *Hippophae rhamnoides*.

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