



ADVANCE STUDY OF POLYPHENOLS IN FOOD PLANTS: SEABUCKTHORN SPECIES AND THEIR RELEVANCE IN HUMAN HEALTH

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ABSTRACT

Aimed of the study of *Hippophae rhamnoides* L berries was isolate and characterize flavonoid compounds. It's belongs to family of elaeagnaceae. It is commonly called as buckthorns. The complete plant of seabuckthorn and especially its fruit berries are source of bioactive compounds. *Hippophae rhamnoides* L berries are one of the best sources of natural antioxidant and its examine to total polyphenol and antioxidant efficacy. *H. rhamnoides* berries were analyzed Phytochemical analysis and chromatographic separation of methanolic extract. It's berries are estimated for the properties of antioxidant, antidiabetic, anticarcinogenic, cardioprotective, antibacterial and anti-inflammatory, antiatherogenic, hepatoprotective, immunomodulatory, antiviral, effects. High concentrations of total phenolics and strong antioxidant activity results showed in the fruits of *H. rhamnoides*

berries. Linear correlation between TPC and DPPH suggested that berries natural source of antioxidants were noted in results.

KEYWORDS: *Analytical characterization, flavonoids and antioxidant efficacy, H. rhamnoides berries, nutritional value, total phenols.*

NOMENCLATURE: The mean values $\pm$ standard deviation, IC_{50} (Inhibition concentration), λ_{max} - The wavelength at which a substance has its strongest photon absorption.

1. INTRODUCTION

Wild edible fruits are consumed frequently by rural and tribal communities across the world and often make an important contribution to the diet and offer affordable means of nutrition

to the people living around. Nutritional and healthcare potential of forest foods explored sporadically is insufficient looking to the wealth and traditional knowledge that exists. NW Himalayan region is one of the biodiversity rich regions of India. The region provides a repository of wild edible plants (Forest Foods). Due to unique climatic and geophysical conditions, plants of the region offer greater possibility of having high quantity of bioactive compounds. Wild fruits offer a convenient but cheap means of nutrition providing adequate nutrient supplies around the living people. *Hippophae rhamnoides*, plant belonging to the family *Elaeagnaceae* and commonly known as Seabuckthorn. Its length of 2-5m and Seabuckthorn is such a one plant that grows wild in temperate part of Asia and Europe.^[1] In India, it is found at high altitude of 2500-4000 m in Ladakh, Jammu & Kashmir, Sikkim and Arunachal Pradesh states of the country.^[3] The chemical composition of *H. rhamnoides* berries include high levels of vitamins C, tocopherols and carotenoids especially β -carotene, lycopene, zeaxanthine.^[4]

Seabuckthorn berries is a rich source of bioactive substances like isoflavones and flavonoids, bioactive compounds have various beneficial effects on health, such as anti-atherogenic, antioxidant, anticancer, and antibacterial effects.^[5] Wild edible fruits are consumed frequently by rural and tribal communities across the world and often make an important contribution to the diet and offer affordable means of nutrition to the people living around. Nutritional and healthcare potential of forest foods explored sporadically is insufficient looking to wealth Medicinal Value and traditional knowledge that exists. *H. rhamnoides* berries have an important source of natural antioxidants activity or Polyphenols they can play vital role in preventing from degenerative diseases. It's using varying polarity solvents and to evaluate the total polyphenols contents of relevant extract and respective antioxidant activity. The study also intended to isolate and characterized certain major flavonoid compounds from *H. rhamnoides* berries.^[6, 7, 8]

2. MATERIALS AND METHODS

2.1 Chemical Analysis

Evaluate to the chemically analysis and solvents use for the extraction of *H. rhamnoids* berries were chromatography, phytochemical screening and in-vitro bioassay of. Folin-Ciocalteu reagent, Gallic acid, DPPH was of analytical grade.

2.2 Plant Material

H. rhamnoids Berrie seeds were separated from the preserve berries by pressing and berries pulp portion was dried at room temperature and used for further extraction.

2.3 Extraction

H. rhamnoids were dried berries pulp and powdered of (100g). It was successively extraction of *H. rhamnoids* berries with n-hexane, chloroform, ethyl acetate, acetone and methanol. *H. rhamnoids* berries keeping it for 24 hrs with intermittent shaking. All extractive values were determined on dry extract weight and ground berry pulp taken weight. The result showed crude extracts were transfer into airtight sample bottles and kept at 4°C.

2.4 Qualitative Phytochemical analysis The qualitative phytochemical screening of the different extracts of *H. rhamnoides* berries showed the presence of alkaloids, steroids, terpenoids, flavonoids, phenolics, tannins, saponins, glycosides, carbohydrate, protein and amino acids in different extracts as presented in Table 2. *H. rhamnoides* berries were prepared and subjected to phytochemical screening to detect the presence and or absence of various phytochemicals constituent.^[9, 10]

2.5 Evolution of total phenolic content (TPC)

The total phenolic concentration of phenolic compounds in extracts was determined spectrometrically using Folin-Ciocalteu method which is widely used for determination of total phenolics. This assay relies on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybdic/phosphotungstic acid reagent to form blue colored complexes that are determined spectrophotometrically at 760 nm.^[11, 12] Methanolic solutions of the chloroform, ethylacetate, acetone and methanol extracts in the concentration of 1 mg/ml were used for the analysis. The solution was prepared by mixing 0.5 ml of methanolic solution of extracts, 2.5 ml of 10% F-C reagent dissolved in water and 2.5 ml of 7.5% Na₂CO₃. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% F-C reagent dissolved in water and 2.5 ml of 7.5% Na₂CO₃. The samples were after incubated in a thermostat at 45°C for 45 max) was determined using spectrophotometer at 760 nm. The samples were prepared in triplicate for each analysis. The absorbance (and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of Gallic acid and the calibration line was construed. Calculation of total phenolic content (TPC) in each extract was made with respect to the equation of the standard curve of Gallic

acid. The total phenolic content was expressed as mg of Gallic acid equivalents (GAE)/g of extracts.

2.6 In-vitro antioxidant assay

The antioxidant activity of *H. rhamnoides* berries was evaluated using a modified 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay.^[13,14,15] The free radical inhibition potential of any compound depends on its ability to donate hydrogen or free electrons. DPPH molecule is a stable free radical with typical purple colour in the presence of an antioxidant, which can donate an electron to it. The change in absorbency is spectrometrically measured at 517 nm. Stock solutions of all extracts were prepared in methanol to achieve the concentration of 1 mg/ml. sixteen serial dilutions of the extract stock solutions and one blank sample (control) was made in methanol. 2 ml of freshly prepared 1 mg/ml stock solution of DPPH was added to each test tube. All the samples were thoroughly mixed and incubated in the dark at 28-300 °C. The absorbance (A) was read at 517 nm after 25 minutes. The percentage of DPPH radical scavenging was calculated using the following formula:

$$\text{Radical scavenging (\%)} = [(Ac - As / Ac) \times 100]$$

Where

Ac - absorbance of the control

As- absorbance of the sample

Lower absorbance values show higher free radical scavenging activity. Ascorbic acid was used as a reference standard in different concentrations (1.46, 3.11, 6.15, 12.4, 24 and 48 µg/ml). The 50% inhibitory concentration value (IC₅₀) is indicated as the effective concentration of the sample that is required to scavenge 50% of the DPPH free radicals.

3. RESULTS

3.1 Extracts values

H. rhamnoides berries of the extract values in n-hexane, chloroform, ethyl acetate, acetone, and methanol are presented in the Table 1.

Table 1: Extract values of *H. rhamnoides* berries with different solvents.

Solvents	Extract Yield (% w/w)
n-Hexane	3.31
Chloroform	4.54

Ethyl acetate	9.55
Acetone	7.20
Methanol	14.22

The percentage yields of n-hexane, chloroform, ethylacetate, acetone and methanol were found to be 3.31, 4.54, 9.55, 7.20 and 14.22 respectively. Methanol extract as solvents showed high removability followed by ethylacetate and acetone. These values presented in Table 1 clearly indicated.

3.2 Phytochemical screening

The results of qualitative phytochemical analysis are summarized in the Table 2. It's presence of carbohydrate, protein, amino acids, steroids, phenolics, flavonoids, tannins, saponins, glycosides and fatty acid found in fatty oil in the berries of *H. rhamnoides* Berries.

Table 2: Phytochemical screening of Extracts of *H. rhamnoides* Berries.

Phytochemicals	n-Hexane	Chlorofom	EtOAc	Acetone	Methanol
Fatty oil	+	–	–	–	–
Steroids	+	–	+	–	+
Terpenoids	+	+	+	+	+
Phenolics	–	+	+	+	+
Flavonoids	–	+	+	+	+
Saponins	–	–	–	–	+
Tannins	–	–	–	+	+
Carbohydrates	–	–	–	–	+
Glycosides	–	–	–	–	+
Protein	–	–	–	–	+
Amino acids	–	–	–	–	+

(+) Present; (–) Absent

Fatty acids found in fatty oil have an important role in several body metabolic and structural functions. Already mentioned in this study, a number of studies have let out that fatty acids are very assorted.^[16, 17]

3.3 Total phenolic Assay

Total polyphenolic content (TPC) of *H. rhamnoides* berries extracts was determined in the given Table 3. By different polarity solvents like chloroform, ethylacetate, acetone and methanol.

Table 3: Total phenolic Assay in different solvent extracts.

Different Solvent Extracts	Total phenolic content (mg GAE/100 mg of extract)
Chloroform	7.93 ±0.13
Ethylacetate	25.44±0.19
Acetone	35.55±0.26
Methanol	49.01±0.24

The total phenolic content in chloroform, ethylacetate, acetone and methanol extracts were found to be 7.93±0.13, 25.44±0.19, 35.55±0.26 and 49.01±0.24 mg of GAE/100mg was recorded respectively. The values of TPC differed among different extracts of *H. rhamnoides* berries. Methanol extract exhibited the highest concentration of Total phenolic content compared by extracts. Lowest TPC was recorded in chloroform extract.

3.4 Analyzed of In-vitro antioxidant assay

The free radical scavenging capacity of the chloroform, acetone and methanol extracts of *H. rhamnoides* berries were determine following modified 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging protocol and the results are expressed as IC₅₀ (µg/ml) are shown in Table 4.^[18]

Table 4: Analyzed of In-vitro antioxidant assay of different extracts.

Different Solvent Extracts	Free radical scavenging Efficacy IC ₅₀ (µg/ml)
Chloroform	331.26±0.33
Ethylacetate	294.21±0.23
Acetone	207.24±0.18
Methanol	123.35±0.24

The DPPH radical scavenging capacities of *H. rhamnoides* berries in solvents extracts like chloroform, ethylacetate, acetone and methanol, expressed as IC₅₀ value were recorded to be 331.26±0.33, 294.21±0.23, 207.24±0.18 and 123.35±0.24 µg/ml respectively. A higher free radical scavenging capacity is connected with a lower IC₅₀ value. The radical scavenging activity is varying according to the polarity vis-à-vis extractability of the solvents. Methanol extracts have the strongest free radical scavenging capacity in the results show.

4. DISCUSSION

4.1. Extractive values

H. rhamnoides berries determined under successive extraction (Table 1) provided Extractive values of idea about extractability of chemical constituents of berries in different organic solvents. The methanol extractive value showed that greater as compared with other solvents.

Plants behavior of different solubility in various solvents extracts. The phytochemicals analysis of best solvent extracts. It is shows that table 1 highest amount of phytoconstituents in successive extraction of methanol yielded.

4.2 Qualitative phytochemical analysis

The successive extraction of *H. rhamnoides* berries of qualitative phytochemical test of the different extracts like n-hexane, chloroform, ethyl acetate, acetone and methanol extracts through declared the presence carbohydrate, protein, amino acids, steroids, alkaloids, terpenoids, phenolics, flavonoids, tannin, saponins, glycosides and fatty oil. Remarkably, phenolics and flavonoids were present in all the extracts except n-hexane.

4.3 The total phenolic content

The values of TPC differed among different extracts of *H. rhamnoides* berries. TPC was recorded lowest in chloroform extract. Highest TPC recorded in the *H. rhamnoides* berries extracts were acquired methanolic extract manifested greater power of phenolic compounds. That's way *H. rhamnoides* berries regard to the nutritional benefits in human life.

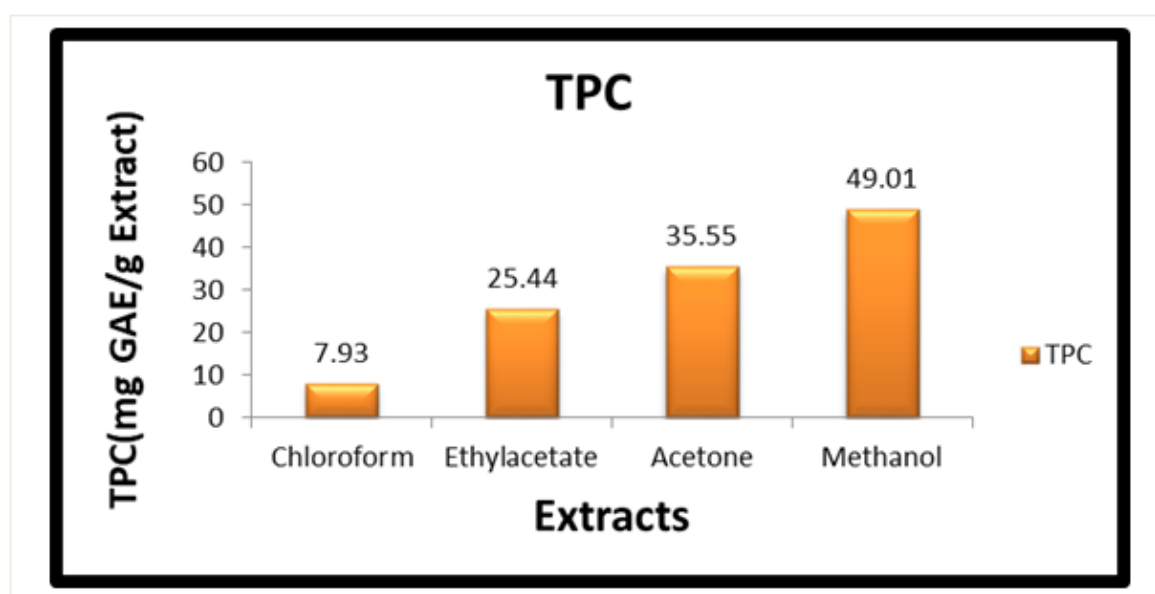


Fig. 1: TPC of different solvent extracts of *H. rhamnoides*.

4.4 The DPPH radical scavenging capacities

The extracts at the used effect of DPPH radical scavenging activity as percentage of free radicals inhibition (Fig. 2)

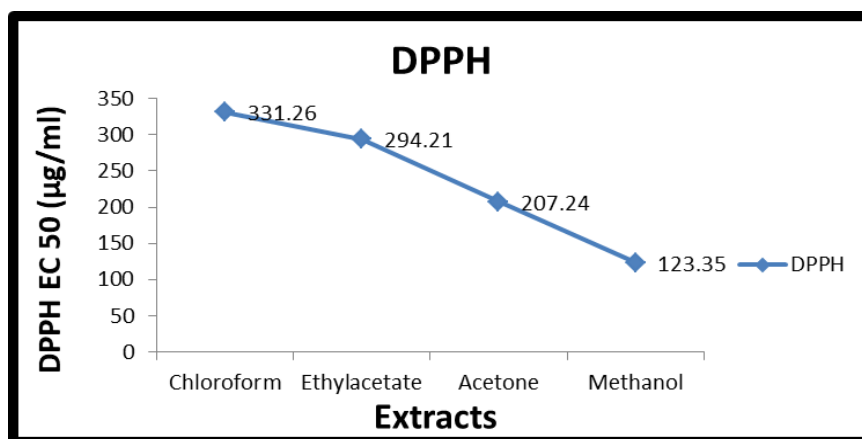


Fig. 2: DPPH of different solvent extracts of *H. rhamnoides*.

4.5 The correlation between the TPC and DPPH-RS of *H. rhamnoides* berries

In Fig.3 shows that correlation between the TPC and DPPH-RS of *H. rhamnoides* berries of different solutions extracts. Higher DPPH radical scavenging capacity is correlated with a lower IC₅₀ value. Free radical scavenging activity is varying according to the polarity vis-à-vis extractability of the solvents. The strongest free radical scavenging capacity shows in methanol extract.

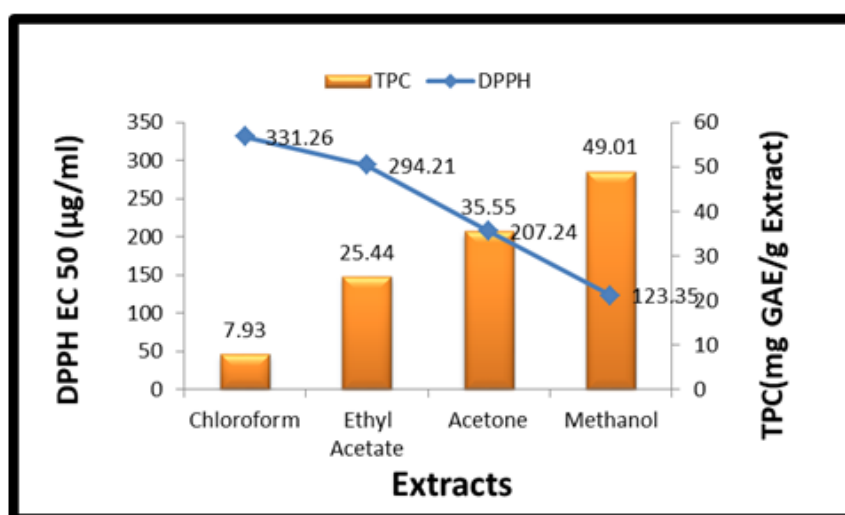


Fig. 3: (Fig.1 and Fig.2) Correlation between TPC and DPPH-RS of *H. rhamnoides* berries.

4.6 Chemistry & Pharmacology *H. rhamnoides*

The *H. rhamnoides* berries include high levels of vitamins C, tocopherols and carotenoids especially β -carotene, lycopene, zeaxanthine, other vitamins such as B₁, B₂, folic acid and large amounts of sugars, mainly glucose and fructose as well as oxalic citric and tartaric acids in chemical composition. In addition of *H. rhamnoides* berries have also noted other chemical

compounds including unsaturated fatty acids, polyphenols, phytosterols, flavonoid, triterpenoids, etc. In the Pharmacological studies have confirmed anti-atherogenic, hypocholesteromic, hypotensive, anti-inflammatory properties, hepatoprotective, antilipemic, antiarrhythmic, antidiabetic, immunoprotective, antimicrobial, cutaneous wound, burn wound healing, antigestrourlcerative, antitumoral, anticancerous and invigorating blood circulation these properties of the *H. rhamnoides*.^[3]

5. CONCLUSION

In the ongoing study, efficacy of berries in relation to its chemical composition is established. In this study the extreme free radicals production generally leads to oxidative stress. *H. rhamnoides* berries have natural anti-oxidants in the ability to protect organisms from damage caused by free radical-induced oxidative stress. *H. rhamnoides* berries have been traditionally used in folkloric medicine and most of the medicinal properties. Also *H. rhamnoides* berries have been attributed to its antioxidant activities. Now considering, this study revealed that *H. rhamnoides* berries has higher polyphenolic content and antioxidant activity. Therefore, it can be ventured that *H. rhamnoides* berries can also be potentially useful as a natural source of antioxidant or in medicine.

As well as phytochemical studies and in-vitro antioxidant assay of fruit Sea buckthorn species will notably add to the inventory of natural antioxidants.

CONFERENCE PAPER

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CONTRIBUTIOR

Dr. Nishat Anjum, Assistant Professor in Chemistry Department, RBBSU, Dehradun carried out all analysis work, noted data, Interpreted the results and composed the manuscript. Visualized the work, designed experimental elements, interpretation and data analysis.

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