Section A-Research paper

PRELIMINARY PHYTOCHEMICAL SCREENING OF *HIPPOPHAE RHAMNOIDES L.* BERRIES OBTAINED FROM NORTHERN INDIA AND ITS ANTIOXIDANT EFFECT

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ABSTRACT

The demand for natural sources in the therapeutic industry is palpably high. Plant extracts are acknowledged as better alternatives to synthetic ones as they are known to cause minimal environmental impact and danger to consumers. The present study focuses on the phytochemical analysis and antioxidant properties of *Hippophae rhamnoides L*. The plant is acknowledged for its many qualities that make it stand out. It is no surprise that it is used in different parts of the world for its nutritional and medicinal properties. Notably, approximately 90 phytochemical agents have been identified so far. However, there are various species of a plant depending on the region they are taken from, and the plant constituent, thus, may vary in attributes. It is a well-known fact that various pharmacological activities concerning the constituent have already been reported, and their results have been striking. It is strikingly challenging to identify the properties of a plant. Notwithstanding the complexities, identification of the properties is essential, as a plant can be put to its best use only after these properties have been identified through screening procedures; the present paper delves into these procedures. The plant source for the present study is Himachal Pradesh, and various constituents have been identified and studied. To examine the properties FTIR and qualitative tests have been duly conducted, and their results have been studied in detail. The FTIR analysis of the crude extract of the plants gives information about the distribution of functional groups. The FTIR and qualitative tests have helped the study be holistic in scope and reliable. The paper seeks to form a basis for further Pharmacological Research.

Keywords: Phytochemical screening, Sea buckthorn, Antioxidant, Pharmacological effect, FTIR

Introduction

Phytochemicals (Greek: phyton = plant) are chemical compounds naturally present in the plants attributing to positive or negative health effects [1]. The richest bioreservoirs of different phytochemicals are found in medicinal plants that are used to treat various illnesses and disorders. The phytochemical components of plants determine their therapeutic qualities.[2]. Alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes, and other essential phytochemicals are found in diverse plant sections.[3]People can harness the metabolites that plants make to protect themselves from biotic and abiotic stresses to create medicines to cure a variety of ailments.[4,5]Several extraction methods can be used to isolate phytochemicals from the plant's constituents. Maceration, percolation, infusion, digestion, decoction, hot continuous extraction (Soxhlet extraction), and other conventional methods are the most frequently used; however, more recently, environmentally friendly techniques like ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extractions (SFE), and accelerated solvent extraction (ASE) have also been introduced. [6,7]Water, ethanol, methanol, acetone, ether, benzene, chloroform, and other types of solvents are employed in the extraction procedure.[8]Pre-extraction conditions have an impact on phytochemical extraction from plant materials (plant part used, its origin and particle size, moisture content, method of drying, degree of processing etc.) There are key variables associated to extraction like extraction method adopted, solvent chosen, solvent to sample ratio, pH and temperature of the solvent, and length of extraction).[6,8]The ability to identify phytoconstituents in plant material aids in the prediction of that plant's probable pharmacological effect.[9]Characterizing and assessing plants and their phytoconstituents allows researchers to investigate the evidence for the medicinal claims made for such plants against a variety of diseases. For the qualitative and quantitative detection of phytoconstituents, advanced techniques such as Gas Chromatography (GC), Liquid Chromatography (LC), High-Performance Liquid Chromatography (HPLC), High-Performance Thin Layer Chromatography (HPTLC), etc. are particularly helpful.[1,8]Nonetheless, conventional phytochemical tests, which are economical, simple, and need fewer resources, remain a reasonable choice for basic phytochemical screening when these techniques are unavailable or prohibitive.[2]

Hippophae rhamnoides L.

In the family Elaeagnaceae, the Sea buckthorn (*Hippophae rhamnoides*) is a deciduous shrub that grows naturally over a wide area in temperate climates. The plant can be found in few places in India such as Ladakh and Lahaul and Spiti deserts, where it is known by a variety of regional names, including Shangti, Dhurchuk, Chhurmak, Sirmaa, and Leh berry.[11]The medicinal substances flavonoids, carotenes, volatile oils, carbohydrates, vitamins, amino acids, and mineral acids are all plentiful in sea buckthorn berries.[12]Sea buckthorn berries' flavonols have demonstrated antioxidant abilities. The goal of the current study was to look at the chemical elements of sea buckthorn berries that might be involved in their antioxidant activity.

Materials and methods

Plant and chemicals

The northern regions of Himachal Pradesh were chosen as a source to harvest Sea buckthorn berries. The collected sample of berries was sent at Raw Materials Herbarium and Museum, Delhi (RHMD) for identification and certificate for crude drug sample authentication. The identification was done on the basis of macroscopic studies of the sample followed by detailed scrutiny of literature and matching the sample with authentic sample deposited in RHMD. It was found correct as berries of *Hippophae rhamnoides L.* which is commonly known as Sea buckthorn (Authentication No.- NIScPR/RHMD/Consult/2022/4156-57). The berries were then ground into a fine powder after being shade-dried and stored.

Aqueous extract

500 ml of distilled water were used to extract the 250 g of powdered dried sea buckthorn berries. Four days of maceration at room temperature ((25˚ ± 2.5˚C) were spent. At low pressure, the solvent was drained out, and the residue was weighed.

Preliminary phytochemical screening

The numerous phytoconstituents found in the berries were tested using the produced extract. Various chemical reagents were made, and specific tests were carried out for particular phytochemicals. Because of their qualitative nature, these assays are known as phytochemical screening. The tests were carried out in accordance with the established practises based on published articles. [13-15]

Table 1: Preliminary qualitative phytochemical tests[10]performed for the detection of different phytoconstituents

Sr. No.	Test	Procedure	Observation
	For Detection of Alkaloids		
1.	Dragendroff's/ Kraut's test	Few mL of extract + 1-2 mL Dragendorff's reagents	A reddish-brown precipitate
2.	Hager's test	Few mL of extract + 1-2 mL Hager's reagents	A creamy white precipitate
3.	Mayer's/ Bertrand's/ Valser's test	Few mL of extract + 1-2 drops of Mayer's reagent (Along the sides of test tube)	A creamy white/yellow precipitate
4.	Wagner's test	Few mL of extract + 1-2 drops of Wagner's reagent (Along the sides of test tube)	A brown/reddish precipitate
	For Detection of Carbohydrates		
1.	Molish's test	2mL of extract + 2 drops of alcoholic α–naphthol + 1mL conc. H2SO4 (along the sides of test tube)	A violet ring

	For Detection of Reducing Sugars		
1.	Benedict's test	0.5mL of extract+ 0.5mL Benedict's reagent + Boiled for 2 min.	Green/yellow/red colour
	For Detection of Glycosides		
2.	Modified Borntrager's test	Plant extract + ferric chloride solution + boil for 5min. + cooled + equal volome of benzene + benzene layer is separated + Ammonia solution	A rose-pink to blood red coloured solution
	For Detection of Proteins and Amino Acids		
1.	Biuret test	2mL of extract + 1 drop of 2% copper sulphate sol. + 1mL of 95% ethanol + KOH pellets	A pink coloured sol. (in ethanolic layer)
3.	Ninhydrin test	2mL of extract + 2 drops of Ninhydrin solution (10mg ninhydrin + 200mL acetone)	A purple coloured sol. {Amino acids}
	For Detection of Flavonoids		
1.	Alkaline reagent test	1mL extract + 2mL of 2% NaOH solution (+ few drops dil. HCl)	An intense yellow colour, becomes colourless on addition of diluted acid
2.	Lead acetate test	1mL plant extract + few drops of 10% lead acetate solution	A yellow precipitate
5.	Ferric chloride test	Extract aqueous solution + few drops 10% ferric chloride solution	A green precipitate
9.	Conc. H2SO4 test	Plant extract + conc. H2SO4	An orange colour
	For Detection of Phenolic Compounds		
2.	Ferric chloride test	Extract aqueous solution + few drops 5% ferric chloride sol.	Dark green/bluish black colour
4.	Lead acetate test	Plant extract is dissolved in 5mL distilled water + 3mL of 10% lead acetate sol.	A white precipitate
5.	Ellagic Acid Test	Plant extract aqueous solution + 5% glacial acetic acid + 5% sodium nitrite solution	Solution turns muddy / Niger brown precipitate
	For Detection of Tannins		
3.	10% NaOH test	0.4mL plant extract + 4mL 10% NaOH + shaken well	Formation of emulsion

			{Hydrolysable tannins}
5.	Lead sub acetate test	1mL extract + 3 drops of lead sub acetate solution	A creamy gelatinous precipitate
	For Detection of Saponins		
1.	Foam test	0.5gm plant extract + 2mL water (vigorously shaken)	Persistent foam for 10 min.
3.	Haemolysis test	Drop of fresh blood on glass slide + plant extract	Zone of hemolysis
	For Detection of Phytosterols		
1.	Salkowski's test	Extract dissolved in chloroform + few drops of conc. H2SO4 (Shaken well and allowed to stand)	Red colour (in lower layer)
2.	Libermann-Burchard's test	50gm extract is dissolved in 2mL acetic anhydride + 1-2 drops of conc. H2SO4 (along the side of test tube)	An array of colour change
3.	Acetic anhydride test	0.5mL plant extract + 2mL of acetic anhydride + 2mL conc. H2SO4	Change in colour from violet to blue/green
	For Detection of Carotenoids		
1.	Carr-Price reaction	10mL extract evaporated to dryness + 2-3 drops of saturated solution of antimony trichloride in chloroform	A blue-green colour eventually changing to red
	For Detection of Carboxylic acid		
1.	Effervescence test	1mL plant extract + 1mL sodium bicarbonate solution	Appearance of Effervescence
	For Detection of Coumarins		
1.	NaOH paper test	0.5gm moistened extract is taken in test tube, mouth of test tube is covered with 1N NaOH treated filter paper, heated for few min. in water bath	Yellow fluorescence from paper under the UV light
2.	NaOH test	Plant extract + 10% NaOH + Chloroform	A yellow colour
	For Detection of Volatile Oils		
1.	Fluorescence test	l0 mL of extract, filtered till saturation, exposed to UV light	Bright pinkish fluorescence

{ }=Indicates presence of specific phytoconstituents

Fourier transform infrared spectrophotometer (FT-IR)

The most effective instrument for determining different kinds of chemical bonding (functional groups) is probably FT-IR. In this annotated spectrum, the wavelength of light absorbed is indicative of the chemical bond. It is possible to identify the chemical bonds of a molecule by reading the infrared absorption spectra. For instrumental analysis, dried plant extract powder was taken into consideration. To create translucent sample discs for the FT-IR study, 10 mg of dried powdered plant extract was encapsulated in 100 mg of KBr pellet. The powdered extract sample underwent FTIR spectroscopy treatment (Shimadzu, IR Affinity 1, Japan).[18] The characterization of drug by FTIR is to detect the any impurity in drug was ascertained by FTIR.Fourier transform infra-red spectroscopy (FTIR) spectrum is characteristic property of a drug for its identification. It is used to identify the functional groups in the molecule. The drug and drug-excipient mixture was scanned at 4 mm/s at 0.5 cm -1 resolution over a wave number region of 400 to 4000 cm -1. The characteristic peaks at particular wavenumber were recorded and checked for characteristic functional groups present in molecular structure.[19]

Antioxidant activity

Utilizing the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Procedure, the antioxidant activity of the berry extract was ascertained.[16]As a standard, butyl hydroxyl toluene (BHT) was used. The extract's absorbance value was determined at 517 nm using the DPPH technique. After measuring the decrease in absorbance at 517 nm, the proportion of radicals that were scavenged was estimated, and then the IC_{50} value.

Result and Discussion

Phytochemical screening

The phytoconstituents discovered in the berry extract included alkaloids, flavonoids, terpenoids, carbohydrates, phenolic compounds, saponins, tannins, glycosides, coumarin, and steroids (Table 2).The extract's contents may have benefits like antioxidant, cytoprotective, cardioprotective, wound-healing, immunomodulatory, improving microcirculation in skin, regulating sleep, appetite, learning, and memory, according to the literature and the analysis done with the results.

Table 2: Summary of Phytochemical Screening of Extract

Sr. No.	Compound class	Result
1.	Alkaloid	++
2.	Carbohydrates	+
3.	Reducing sugar	-
4.	Glycosides	+++
5.	Proteins and amino acid	-
6.	Flavonoids	++
7.	Phenolic compound	+++
8.	Tannins	++
9.	Saponins	+++
10.	Phytosterols	++
11.	Carotenoids	-
12.	Carboxylic acid	-

13.	Coumarins	+++
14.	Terpenoid	+
15.	Volatile oil	-

+ Indicated present, ++ indicated moderate present, +++ indicated high present, - indicated absent

FTIR Interpretation

The FTIR spectra of dry extract is shown in the figure 1. The principal IR absorption peaks of dry extract have the respective characteristics such as at 1785.82 cm -1 appears due to the stretching vibrations of carboxylic groups (C=O) in the volatile oils, triglycerides, aliphatic esters or other compounds in the extracts, 1662.56 cm -1 can be assigned to the conjugated carbonyl bonds from flavonoids, 1356.96 cm -1 band is due to the β-ionone ring of β -carotene or due to the CH (–CH 3) symmetrical bending, 1148.07 cm -1 are specific for carbohydrates with C-O and C-OH stretching vibrations, 1075.61 cm -1 are assumed to be produced by the C-O stretching of the ester group due to reduced carbohydrates content followed by extraction procedure and 703.13 cm -1 is probably due to the methylene CH 2 rocking band from long CH 2 chains. These observed principal peaks confirmed the purity and authenticity of the dry extract that it has been extracted from *Hippophae rhamnoides* dry fruit.

Figure 1: FT-IR spectra of Dry Extract

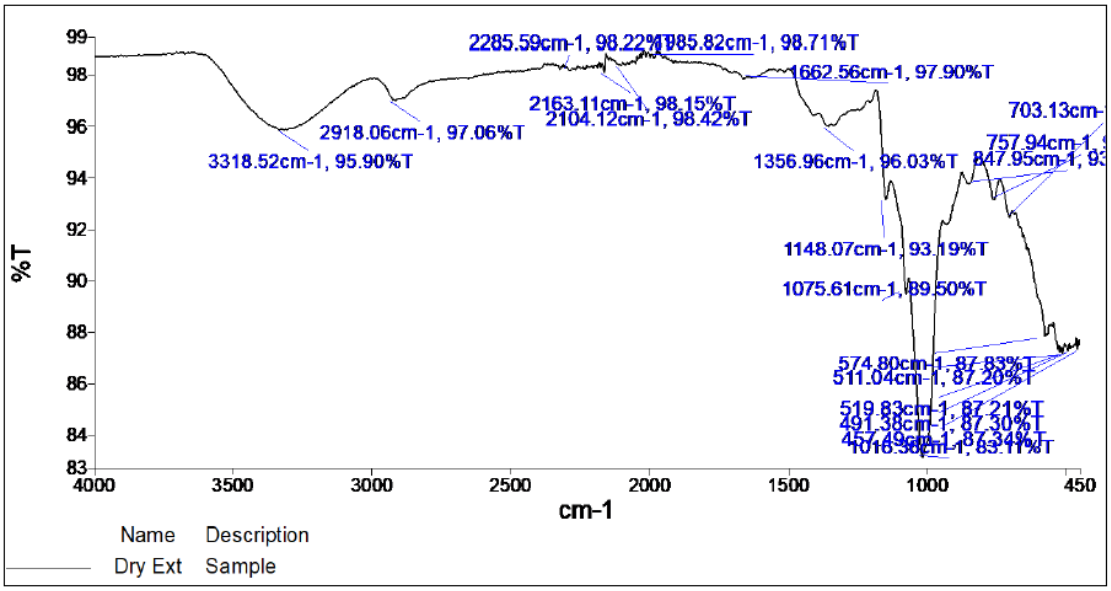

Table 3: FTIR Interpretation of Dry Extract

S.No.	Functional Group	Reported (cm^{-1})	Observed (cm^{-1})
1.	vibrations of carboxylic groups (C=O)	1743	1785.82
2.	conjugated carbonylbonds	1622	1662.56
3.	CH ($–CH_3$) symmetrical bending	1377	1356.96

4.	C-O and C-OH stretching vibrations	1170-930	1148.07
5.	C-O stretching ofthe ester group	1161, 1116 & 1093	1075.61
6.	Presence of methylene CH_2 rocking band from long CH_2chains	721	703.13

Antioxidant activity

The standard BHT demonstrated scavenging activity against DPPH (IC50 0.035±0.01) and the aqueous extract demonstrated antioxidant potential (IC50 2.24±0.02). A compound's ability to function as an antioxidant depends on its molecular structure and where the hydroxyl and pernyl groups are located.[17]In the DPPH technique, the aqueous extract demonstrated good antioxidant activity. The preliminary screening of antioxidant potential is done using the more reliable and time-efficient DPPH radical scavenging approach.

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