
Studies on the Phytochemistry, Medicinal Properties of Hippophae Rhamnoides

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Abstract

Because of its anti-inflammatory, antibacterial, and anti-cancer capabilities, Hippophaerhamnoides L. of the Elaeagnaceae family has been known as the "wonder plant." In addition, it shields the body against rheumatoid arthritis, psoriasis, multiple sclerosis, and other conditions that affect the throat, lungs, skin, and cardiovascular system. Seabuckthorn grows in five states in India: Himachal Pradesh, Sikkim, Jammu and Kashmir, Utrakhand, and Arunachal Pradesh. Two of the four Hippophae species in these five states are in Jammu & Kashmir. Seabuckthorn is abundant in this state's Ladakh area, with populations flourishing in the districts of Leh and Kargil. Because the issue is so essential, the leaves of Hippophaerhamnoides are investigated to discover whether it contains any bioactive compounds. Generally, samples of shade-dried leaves from several Kargil populations are made using one of three distinct organic solvent: acetone, water, and methanol. In other cases, chloroform, meta-phosphoric acid, & petroleum ether were also detected. These were then analysed and subjected to various colour tests. According to the findings, these leaves contain many essential antioxidants and other health-promoting compounds. All three extract types tested positive for ascorbic acid, phenols, tannins, sterols, flavonoids, alkaloids, and saponins. All were devoid of anthocyanides, phlobatannins, steroids, and leucoanthocyanins. The aqueous extract contains terpenoids, flavonols, as well as cardiac glycosides. Their behavior in other solvents, however, is different. This article reviews the research on the medicinal properties of Sea buckthorn (Hippophaerhamnoides L.) for the treatment of both acute and chronic conditions. In many regions of the globe, the plant is eaten and utilised because of its nutritional and therapeutic benefits. Numerous in vitro, in vivo, and even some clinical research have investigated the therapeutic and pharmacological potential of sea buckthorn in the recent past. Several biochemical and pharmacological research has been conducted on sea buckthorn,

Keywords: Hippophaerhamnoides; terpenoids, flavonols, and cardiac glycosides; pharmacological properties; anti-microbial, anti-tumor, and tissue regeneration.

Introduction

Concerns regarding the carcinogenicity of synthetic antioxidants such as butylated hydroxyl toluene (BHT), BHA (butylated hydroxyl anisole), and PG (propyl gallate) have driven an increase in research on antioxidants derived from natural bio-resources in recent years (Branen, 1975). That is possibly why many doctors have used or are using antioxidants derived from natural sources to postpone, reverse, or reduce tumorigenesis (Shureiqi et al., 2000) (Tsao et al., 2004). It has created an incentive to investigate and test taxonomics with a high potential for producing such bioactive components. Seabuckthorn is one example of this kind of bioresource. This multipurpose plant, which contains over 190 bioactive substances (Singh et al., 2006) (Shah et al., 2007), has the potential to support multiple industries. It has also been achieved practically in China, Russia, and others. India, the world's fifth biggest seabuckthorn bioresource in terms of area covered, is also developing in this manner. The country is endowed with an abundance and diversity of germplasm used exclusively for fuel and feed by indigenous people. When the then-FRL (DRDO) Leh employed seabuckthorn to green Ladakh as part of a cold desert tree plantation initiative, its potential in Jammu and Kashmir was recognized in 1992. However, since the capacities of the whole plant's infrastructure have come to light, a medicinal drink made from the plant's

fruits has been developed, patented, and sold under several trade names such as Leh berry, Ladakh berry (Dwivedi et al., 2006). Although the therapeutic use of this herb stretches back over 1,000 years in Amchi, Chinese, Mongolian, and Tibetan traditions (Singh et al., 2006), its usage in the current allopathic system has only lately been recognized. It has been shown to have antibacterial, anti-cancer, anti-inflammatory, and anti-aging effects and is well recognized for all these (Eccleston et al., 2002). A range of antioxidant compounds discovered in various parts of this plant is claimed to confer all of these properties. Because the research published on seabuckthorn in India is lacking in terms of Ladakh germplasm and extensive biochemical studies, the current research was performed with this in mind. Thus, this paper aims to conduct a qualitative investigation of the bioactive chemicals found in the leaves of *Hippophaerhamnoides* gathered in Kargil (J&K, India). Recently, sea buckthorn (*Hippophaerhamnoides* L.) Elaeagnaceae, a rare and precious plant, has garnered international interest primarily due to its therapeutic and nutritional potential. Prickly, deciduous, and nitrogen-fixing, the sea buckthorn is a shrub that is endemic to the dry and chilly regions of Europe and Asia. It is also often referred to by its acronym, SBT. Due to its nutritional and therapeutic benefits, it is presently cultivated in numerous regions of the globe (Li, 2003). It is resistant to drought and frost with aggressive vegetative reproduction and a robust, complex root structure that contains nitrogen-fixing nodules, making it valuable for land restoration and farmstead protection. Although the exact number of species that belong to the *Hippophae* genus is uncertain, experts assume that there are seven different species. In India, three species have been described: *Hippophaesalicifolia*, *Hippophaetibetana*, and *Hippophaerhamnoides*, with *Hippophaerhamnoides* L. ssp. *Turkestanica* is the most important. It is found in its native state in the cold deserts and arid temperate zones of the northern Himalayas (2,590–4,175 meters above mean sea level). Mongolia, Norway, China, Germany, Nepal, Finland, Poland, India, Pakistan, Denmark, Great Britain, Latvia, Romania, Netherlands, France, Russia, Sweden, and Canada are natural distribution areas for sea buckthorn. There are now eight subspecies of *Hippophaerhamnoides*. In Finland and China, respectively, the *Hippophaerhamnoides* L. ssp. *rhamnoides* and *Hippophaerhamnoides* L. ssp. *Sinensis* subspecies are the ones that have been seen and reported the most often. Cultivating SBT in a wide variety of soils and harsh environmental conditions in the temperate areas of Europe and Asia is possible. The SBT is a deciduous, thorny shrub that reproduces by its own stamens. SBT often develops a 3-4 m tall shrub or small tree. Its robust and intricate root structure with nitrogen-fixing nodules and symbiotic relationship with the presence of frankiaactinorhizal fungus in SBT makes it an appropriate pioneer plant for the conservation of water as well as soil in damaged areas. The leaves are alternating, thin, lanceolate, and grey in hue. The male bud is made up of four to six apetalous flowers that produce pollen that is disseminated by the wind, while the female bud is made up of a single apetalous flower with one ovary and one ovule. Female plants yield fruit that resembles berries and measures between 6 and 9 millimetres in diameter. These fruits are tender, delicious, and rich in oil. The mature barriers have an orange/red coloration and a drupe-like appearance; each contains a single seed encased in a tender, fleshy outer layer.

Aims and objectives

- To study the phytochemistry of the plant *H.rhamnoids* by using the solvents.
- To study the different Medicinal properties of *H.rhamnoids* generally.

Objectives

- Preparation of the solvents required for the phytochemical study.
- We are conducting assays respectively for the predicted medicinal properties.

Literature review

(Bhattarai et al., 2010) Three therapeutic plants native to the Nepali Himalayas were studied for their biological effects. The antibacterial, cytotoxic, and antiproliferative properties of methanol extracts of

leaves and branches of herbs obtained at various altitudes in Nepal, Hippophaerhamnoids, Rhododendron lepidotum, as well as Cornuscapitata, are evaluated. The methanolic extract of Rhynchosporium lepidotum has an antibacterial action, and the effectiveness of C. capitata against Staphylococcus aureus is similar to the antibiotic streptomycin; on the other hand, the performance of the H. rhamnoids extraction is insignificant. The cytotoxicity (LC50) readings for R. lepidotum varied from 46.4 to 111.6 g/ml, whereas the cytotoxicity (LC50) readings for C. capitata varied from 129.4 to 464.1 g/ml. The R. lepidotum extract has a high cytotoxic characteristic, while the C. capitata extract has a moderate cytotoxic property. R. lepidotum's antiproliferative activity against the HeLa cell line was equivalent to that of C. capitata and H. rhamnoids to make a comparison. According to the results of the phytochemical research, the bioactivities shown by these plants may be attributable to the existence of steroids, tannins, flavonoids, terpenoids, coumarins, as well as glycosides.

(Yang, 2019) The anti-inflammatory and anti-obesity properties of the leaves of Hippophaerhamnoids were investigated. H. rhamnoids components were separated and purified using various open-column chromatographic methods, including semi-preparative HPLC. The spectroscopic examination provided insight into the structures of isolated substances. Their inhibition of nitric oxide production in RAW264.7 cells were examined, as well as the accumulation of triglyceride in 3T3-L1 cells. Eighteen tannins and other compounds were isolated and identified as 1,2,6-tri-O-galloyl-D-glucopyranose (1), 1,3,6-tri-O-galloyl-D-glucopyranose (2), 1,4,6-tri-O-galloyl-D-glucopyranose (3), and 1,3,4,6-tetra-O-galloyl-D-glucopyranose (4). (S) 1-O-galloyl-2,3-glucopyranose (7), -HHDP-D-glucopyranose (7) (S) -HHDP—D-glucopyranose (8), 1,3-di-O-galloyl-4,6-(S)-HHDP—D-glucopyranose (9), and 1,6-di-O-galloyl-2,3-(S)-HHDP—D-glucopyranose (10). (2,3). (S) -HHDP-D-glucopyranose (11), casuarictin (13), and 1,2,3-tri-O-galloyl-4,6-dione (14). (S) 1,4,6-tri-O-galloyl-2,3- -HHDP-D-glucopyranose (12) (S) – HHDP-D-glucopyranosyl-D-glucopyranoside (13), hippophaenin B (14), pedunculagin (15), casuarinin (16), ellagic acid (17), and pinitol (18). H. rhamnoides leaf tannins exhibited anti-inflammatory and anti-obesity properties. The compounds 2, 3, 5, 6, 8, 10, 12, and 13 were originally isolated from this species. (Bal et al., 2011) Recently, sea buckthorn (Hippophaerhamnoides L.), a primitive plant with contemporary uses, has attracted attention worldwide, primarily due to its nutritional and therapeutic potential. The berries include a wide variety of vitamin, essential fats, free amino acids and elemental components, among other nutrients and bioactive compounds. The review delves into the sustainability and post-harvest management of the crop and gives a concise summary of the connected studies. Current compositional data on medicinal berries is integrated to create a complete database of real information on chemically & medicinally significant elements of diverse sources and varieties. Given the scientific understanding of the importance of the compounds or nutritionally relevant components found in herbal berries, it is clear that medicinal berry is one of the primary sources of these components and should be used as a nutritious alternative in the commercial sector. Sea buckthorn berries from various agroecological zones should be processed in different ways, and the resulting effects on the berry's overall nutritional quality should be thoroughly studied. Consequently, several significant knowledge shortages revealed in this article will stimulate new academic and R&D endeavors, establishing creative job profiles in the food and cosmeceutical sectors.

Materials and methods

The plants were first gathered and processed for the studies such as pharmacognostic and pharmacological studies.

The Accumulation Of Flora

The plant material for the research was gathered in Kashmir. A sample (HR0706/SH18 & HR0707/SH19) was saved in the lab as a future reference.

Chemicals used

Both Sigma Chemical Co. and Lonza, India, graciously provided the DMEM, FBS, streptomycin, penicillin, PI, and dichlorofluoresceindiacetate (DCFDA). Nothing else was less than analytical quality.

Instruments used:

Different-sized photos were taken with an inverted binocular microscope built into a Leica camera. Observations were done in a bright field.

Pharmacognostic study**Macro and microscopic study**

The leaves shape, size, color, and surface were utilized to describe the broad morphological traits. Transverse leaf slices were inspected under a microscope, dyed with phloroglucinol and hydrochloric acid for microscopic analysis, and put through the usual research processes. Particles more significant than a 200-mesh sieve Sodium hydroxide washing and phloroglucinol & hydrochloric acid staining were used to analyze the powder characteristics of the leaf, and then 60 were mounted in a glycerol medium. To ensure the safety and efficacy of herbal remedies, physiochemical analysis was done on powder sample leaf material. As well as extractive values, we also ran tests for fluorescence, moisture, total ash, water solubility, and insoluble ash.

Initial phytochemical testing

The leaves were ground into a powder using a machine, let to dry naturally in the shade, and then passed through a 40-mesh sieve. Hexane, chloroform, ethyl acetate, methanol, & water were used in the cold maceration method to extract the powdered dry material. A vacuum evaporator running at a reduced pressure was used to remove the solvents completely. Numerous phytoconstituent tests, including those for amino acids (Ninhydrin), flavonoids (Shinoda), steroids (Dragendorff), alkaloids (Dragendorff), terpenoids (Liebermann-Burchard), tannin, or phenolic compounds, all returned favourable results (Chloride test) and others.

Medicinal Attributes

Hippophaerhamnoides berries were crushed and progressively extracted. 320 grams were dissolved in a mixture of a 1:1 ratio of distilled water and analytical-grade methanol. The mixture had a volume of 2 liters. Before going through a filter, the plant material that had been masticated was first passed through eight layers of muslin fabric to achieve a coarse filtration. The filtrate was evaporated in a rotary vacuum evaporator set to 40°C and operating at a lowered pressure.

Assaying enzymes

The enzyme test was conducted using the Berthelot assay with a minor modification. Here, it is briefly explored. The 96-well plate had 85 mL of the assay mixture & 10 mL of phosphate buffer (pH 7.0) added to each well. After that came the enzyme solution (25 L) and the sample solution (10 L). This combination was then pre-incubated for five minutes at 37 °C. Following this, each well-received 40 mL of urea standard solution (20 mM), and incubation at 37°C for an additional 10 minutes. After 10 minutes, each well received 115 l of phenol hypochlorite reagents, which were newly made by combining 45 litres of phenol reagent and 70 litres of alkali reagent. Another 10 minutes of incubation at 37°C was added to allow color development. Following that, an absorbance measurement at 625 nm was obtained using the Synergy HT 96-well plate reader [4]. The following formula gave our calculation of the enzyme inhibition rate: Reduction in Activity = 100 - (Test Sample Absorbency / Control Sample Absorbency) 100 Ez-Fit enzyme kinetics software was used to get the IC50 values (concentration at which 50 percent of the total of an enzyme-catalyzed reaction occurs).

production of an extract with antioxidant and neuroprotective properties

The sieved powder was put in an airtight container before being deposited in a sealed jar and maintained at room temperature until further testing was undertaken. Using a rotating evaporator and reducing pressure, the extract was dried. The extract was made in a dark vessel at 20 ° C.

Activity to scavenge DPPH radicals

The ability of plant extracts to scavenge free radicals is often assessed using the stable free-radical molecule test known as DPPH. The extraction methods were dried in a revolving vacuum evaporator with progressively decreasing pressure. The extract was 04 percent DPPH methanolic solution was added to start the assay. After one hour of incubation at 30 degrees Celsius, the absorption at 515 nanometers was recorded using a microplate. Inhibition percent = $100 - 100(A_s / A_o)$, where A_s is the sample's absorbance at 515 nm and A_o is the blank's absorbance, was used to calculate the percentage of inhibition.

Scavenging of hydroxyl radicals in tests

The hydroxyl radical scavenging test was conducted using Su et al. (1998)'s methodology with a few minor modifications. Two milliliters of *H. rhamnoides* extract were mixed with two milliliters each of ferric chlorides (6 millimolar) and hydrochloric acids (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 mg/ml) (6 millimolar). 10 minutes into the incubation period, sodium salicylate, 6 m, 2 ml was added.

It was then placed in an incubator at 37°C for 30 minutes. After an appropriate incubation time, the samples are analyzed using a spectrometer (Lab India), and the maximum reduction is determined with the use of a formula:

Adding sodium salicylate to a sample leads to an absorption spectrum with the formula $1 - (A_s - A_w / A_o) * 100$, wherein is the percent of inhibition. Samples that do not contain salicylates have a fluorescence value of A_w , whereas the reagent has a value of A_o .

FTC evaluation

The ferric thiocyanate test was used. It was observed that *H. rhamnoides* has antioxidant capabilities that help prevent lipid peroxidation. Four milliliters of leaves extract in 70% ethanol, four milliliters of linoleic acid in acetone at a concentration of 2.51%, eight milliliters of phosphatase buffer at a pH of seven, and 3.5 millilitres of dI water were placed in a screw-top vial.

The vial was placed in an unlit oven at 40°C. The solution was dilute with 9.7 mL of 75percentage ethanol and 0.1 mL (30% concentration) of ammonium thiocyanate. The absorption of the resultant combination was measured once daily at 500 nm until the control attained its maximum absorbance. It took three minutes to add 0.03 ml of 0.03 M ferrous chloride to 3.50% hydrochloric acid (HCl). A-tocopherol was utilised as a standard of comparison.

Cellular Line Upkeep

The human neural tissue was donated by the Central Institute for Csms in Pune, India. Cell line IMR32 for purchase. The IMR32 cells grew in a 5% CO₂ incubator at 37 degrees Celsius in DMEM supplemented with 10% Foetal Bovine Serum, penicillin (100 units/ml), & streptomycin (100 units/ml).

Examining how *H. rhamnoides* affects IMR32 directly

To investigate the toxicity spectrum of “*H. rhamnoides*” extracts, the effect was examined prior to the oxidative test.

The MTT test was used to assess the viability of IMR32 cells after they were exposed to various doses of *H. rhamnoides* extract. MTT is a colorimetric test used to determine cell cytotoxicity. Following 24 hours of cultivation in various concentrations of *H. rhamnoides* extract (ranging from 0 to 1000 mg/ml), exponentially growing IMR32 cells were detached from the 96-well plate and analysed. Each well was kept at 37 degrees for 2 hours after it had been given 100 II of MTT at a concentration of 5 mg/ml. Following 10 minutes, the samples were analysed in a plate set to 570 nm, Then, 100 II of DMSO were poured into each well after the solution was drained. (Thermo Scientific, Multiskan).

Using propidium iodide staining, we looked at whether *H. rhamnoides* had any neuroprotective properties against induced oxidative cytotoxicity.

Staining DNA using the fluorescent reagent propidium iodide (PI) allows researchers to study factors such as DNA content, cell proliferation, cell cycle analysis, & DNA damage. After an initial incubation period of 24 hours, the cells were seeded in a 12-well panel at a density of $1 * 10^6$ cells per well. After

that, *H. rhamnoides* extract (at concentrations between 3.2 and 100 g/ml) was added to the cells for 24 hours. It was followed by the cells being subjected to the toxic stress-inducing chemical H₂O₂ for the same period. Using a flow cytometer (FACSCalibur, BD Bioscience), PI was used to impart a stain to the cells and evaluated alongside appropriate controls. The flow cytometer's emission and excitation ranges were ex 535/em 617.

Statistic evaluation

The findings were provided as mean SD values, and each experiment was performed in triplicate. Using the sixth edition of the Graphpad Prism program, Dunnett's experiment was done out employing ANOVA to analyze the data and determine the significance of the differences between the various groups. The value of 0.05 was selected as the statistical significance criterion.

Results

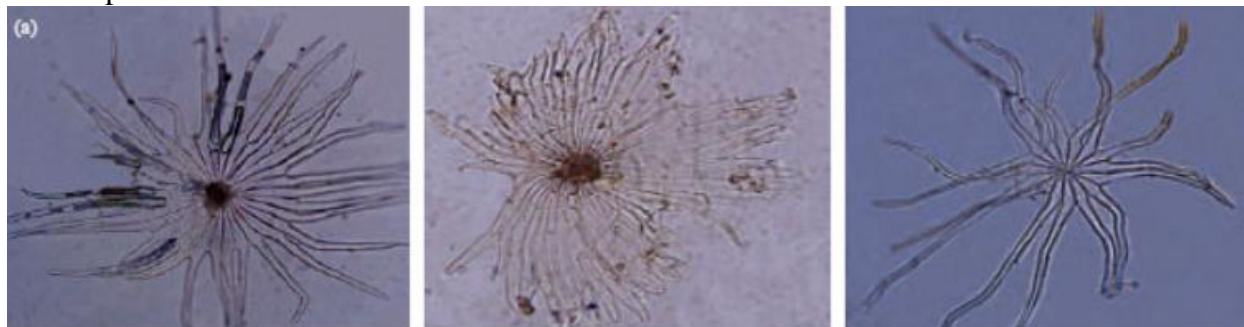
Macroscopy

Leaves are opposite, petiolate, and straight to linear-lanceolate shape, measuring 2-8 x 0.2-0.8 cm (narrower at the base), with a revolute border and a subobtuse tip; the other side has a dark greyish green color.



Microscopy

Powder microscopy showed the existence of many forms of trichomes, included stellate, peltate, as well as a mix of the both stellate-peltatetrichomes, as well as palisade cells and fractured xylem arteries in the wounded epidermis.



Preliminary phytochemical studies

Steroids, saponins, terpenoids, carbohydrates, phenolic chemicals, and flavonoids were discovered during the preliminary phytochemical screening of several leaf extracts from *Hippophae*.

Physical-chemical analysis:

The physical-chemical characteristics that were looked at are included in Table 2, along with their respective results. The total quantity of ash from *H. rhamnoides* (80). Leaf tissue from *H. rhamnoides* has less water soluble ash (4.09). The physiological start making of the leaf is revealed by water-soluble ash. Table 3 shows the results of the extraction values that were performed. To better understand the phytoconstituents present in the leaves, the powder properties of the leaves were studied after being treated with a range of chemicals and then exposed to UV and sunlight.

Table 1. Preliminary studies

Phytoconstituents	<i>H. rhamnoides</i>				
	Hexane	Chloroform	Ethyl acetate	Methanol	Water
Steroids	+	+	+	+	+
Phenolic compounds	-	-	+	+	+
Saponins	-	-	-	+	+
Flavonoids	-	-	-	+	+
Coumarin glycosides	-	-	+	+	+
Alkaloids	-	-	-	-	-
Proteins	-	-	-	+	+
Carbohydrates	-	-	-	+	+

Table 2. Extracted values of *H. rhamnoides* leaf

Solvents	<i>H. rhamnoides</i>
Hexane	2.34
Chloroform	3.32
Ethyl acetate	4.62
Methanol	20.66
Water	19.98

Table 2. Physiochemical characters

Parameters	<i>H. rhamnoides</i>
Total Ash	80
Acid-insoluble ash	10
Water soluble ash	10
Moisture content	7.77
Foaming index (No unit)	500

Inhibitory action for urease

The Berthelot test was used to ascertain the anti-urease action of *H. rhamnoides* methyl as well as ethyl alcoholic extracts. The plant showed more significant inhibition of urease activity. The straight-line equation used to compute the IC50 value of each extract yielded a slope of 0.3654 and an r2 of 0.9269, as well as percentage inhibition values of 91.691.21 (Et-OH) and 93.080.88. (Me-OH)

Assay for scavenging free radicals

In DPPH, ascorbic acid and OH radical scavenging were used as standards, while FTC assays were used to assess *H. rhamnoides*' ability to neutralise free radicals. According to the findings, the level of radical scavenging activity of an *H. rhamnoides* extracts increased with increasing concentration. As can be shown in Fig. 1a, the half-maximal effective concentration for the DPPH assay was 70.81 g/ml. Substantial OH radical scavenging activity was also observed for the extract (Fig. 1b). The current research demonstrates that *H. rhamnoides* is an effective antioxidant since OH radical scavenging

capability is a crucial criterion in assessing antioxidant property. According to FTC data, the extract has similar lipid peroxidation activity compared to conventional.

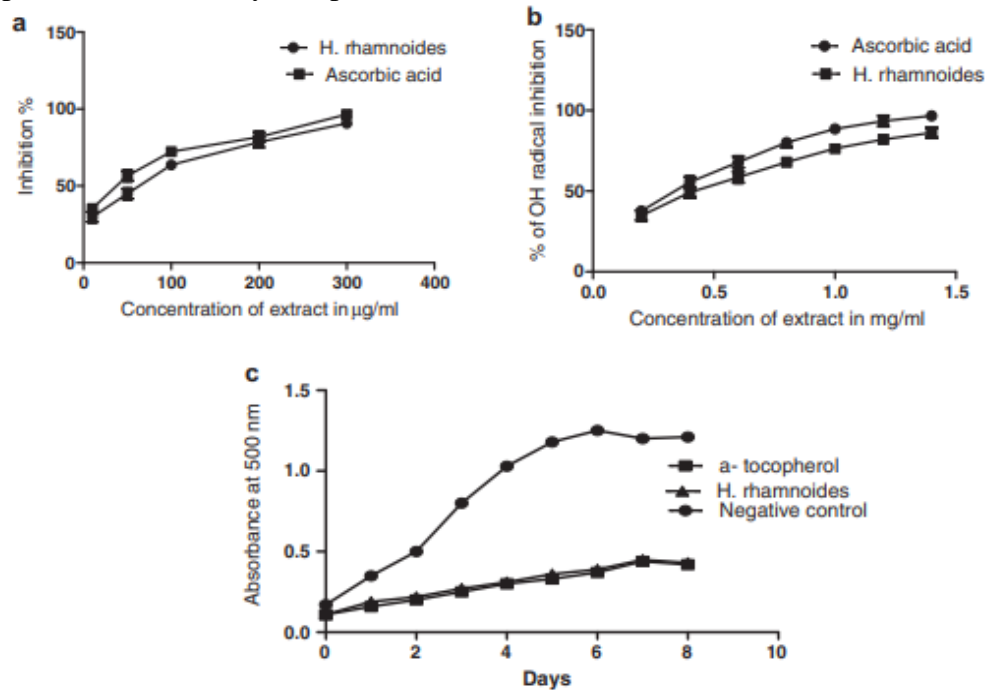


Figure 1 DPPH test (a), OH is scavenging free radicals ability (b), & lipid peroxidation of *H. rhamnoides* (c).IMR32 Cell Response to *H. rhamnoides* Extract.

Researchers assessed the extract's cytotoxicity by incubating the adult brain cell line IMR32 with doses that ranged from 1 to 1000 g/ml (the findings for 1000 g/ml are not displayed). The results are shown in Figure 2. The toxicity test results revealed that a higher extract concentration resulted in a decreased percentage of viable cells. The IC50 value was discovered to be 312.63 2.6 g/ml. Because of this, concentration had the least detrimental effect on cell viability. It was selected as the maximum possible dosage without compromising safety to evaluate the extract's effectiveness.

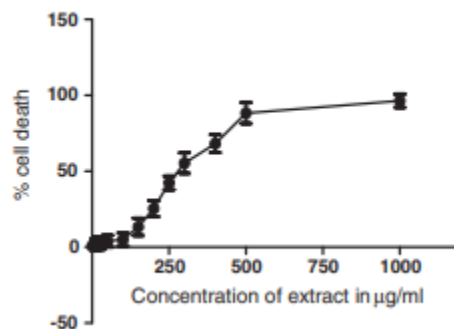


Figure 2 shows that the human brain cell line IMR32 requires *H. step* to operate, and that this activity is concentration dependent. Take notice that the data are shown as mean SD. The results of toxicity experiments on the IMR32 control cells using different dosages of *H. rhamnoides* extracts.

H. rhamnoides has a neuroprotective effect against H₂O₂-induced cytotoxicity.

IMR32 cells were challenged with 250 IM H₂O₂ for 24 hours after being pretreated with varying doses for 24 hours of *H. rhamnoides* extracts (ranging from 3.2 to 100 mg/ml). The neuroprotective action of *H. rhamnoides* was determined by observing how well the cells survived the challenge. The cells' demise was verified by staining them with propidium iodide in an experiment. As indicated in the figure, treated cells had a dose-dependent improvement in cell viability compared to H₂O₂ controls. The ideal

concentration was 100 g/ml, and the MTT tests yielded similar results (results not shown). The plant is effective at a concentration of one-hundredth lg/ml. There was a dramatic rise in the number of healthy cells when the extract was added.

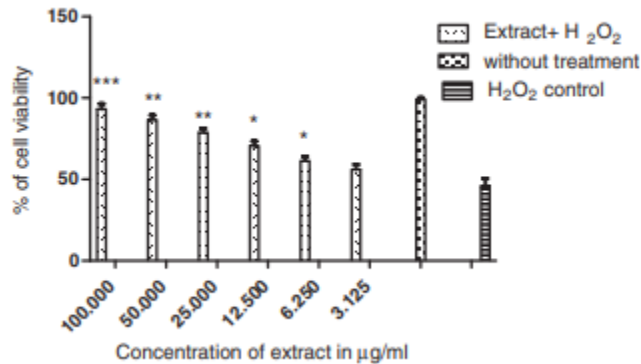


Figure 3. PI staining test for *H. rhamnoides*' neuroprotective impact on H₂O₂-induced cell cytotoxicity. Notice: Values are shown as mean SD. The degree of significance was determined using an ANOVA and the Dunn test. The treated group was contrasted with the H₂O₂ control group. The toxicity tests were performed on the IMR32 cell line after pre-incubation with various doses of *H. rhamnoides* extract at 250 IM H₂O₂.

Conclusion

Hippophaerhamnoids, also known as seabuckthorn, show various pharmacological properties. In this study, we discussed the identification of phytochemical constituents and conducted assays for determining the inhibitory action of urease and *H. rhamnoides*' neuroprotective activity against H₂O₂-induced cytotoxicity.

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