

Photochemical investigation of medicinal plant *Hippophae Rhamnoides*

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Abstract

The sea buckthorn plant (*Hippophae rhamnoides* L.) helps treat a wide range of short- and long-term illnesses. Its therapeutic and pharmacological properties have been extensively studied through the use of many *in vitro* and *in vivo* models. Undoubtedly, the future offers much potential for SBT bioactives. There are 18 distinct kinds of essential amino acids and 24 critical minerals in seabuckthorn juice. In addition to a healthy balance of omega-3 to omega-6 fatty acids, the oil extracted from seabuckthorn seeds is particularly rich in oleic acid. In addition to protecting the skin from harmful UV rays, the oil helps keep the skin healthy. Traditional uses of the plant to heal a wide range of diseases have been confirmed and built upon by recent scientific research. Those in fields as diverse as biotechnology, nutraceuticals, pharmaceuticals, cosmetics, and the environment may all learn something from the seabuckthorn shrub because of its unique and valuable features. Hypertension, edoema, ulcers are just some of the ailments that this plant's berries, seeds, and leaves are used to cure in its traditional folk medicine form. other beneficial chemicals have all been identified via phytochemical analysis. Our research showed that SBL has a lot of valuable nutrients, including protein and minerals. The optimal conditions for organic acid profiling in SB berries were initially established using RP-HPLC-DAD analysis. The plant's beneficial and therapeutic characteristics have been studied intensively for decades. The presence of bioactive substances such as triterpenoids, saponins, and ellagitannins gives *Hippophae rhamnoides* its therapeutic potential.

Keywords: *Hippophae rhamnoides*, Phytochemicals, Sea buckthorn, Medicinal Properties

1. Introduction

To remedy both immediate and chronic health issues, the sea buckthorn (*Hippophaerhamnoides* L.) herb is indispensable. Clinical and pharmacological effects have been studied extensively in a wide variety of *in vitro* and *in vivo* settings. A massive and quickly growing body of experimental evidence demonstrates significant characteristics and bioactive compounds in SBT. Antioxidant, immunomodulatory, anti-inflammatory, anti-atherogenic, cardioprotective, and wound-healing properties are only some of the pharmacological and therapeutic activity shown by SBT's many components (leaves, fruits, and seeds). Undoubtedly, the future offers much potential for SBT bioactives.

Standardized extracts of SBT need to be studied more thoroughly before their effectiveness can be judged. To create a cost-effective, potentially herbal medication on a broad scale, more systematic investigations are required to assess the efficacy using standardized extracts of SBT and to identify the bioactive components responsible for the biological activities. Since plants and plant products vary greatly standardise the formulations based on ingredients (Suryakumar and Gupta, 2011).

The sea buckthorn, or *Hippophae*, is a deciduous shrub or small tree found in many oceanic and coastal regions. Various. Further study is required to elucidate the molecular and cellular mechanisms involved in healing these diseases. Cancer therapy, cardiovascular disease, gastrointestinal ulcer and skin issue treatment, and liver protection are only a few of the major research fields discussed in the current paper (A. Zaib, 2004).

The blooming shrub known as sea buckthorn is indigenous to the cool temperate zones of Eurasia. Hypertension, edoema, , , and ulcers are only some conditions that the plant's berries, seeds, and leaves are used to cure in traditional medicine. About 190 bioactive substances, including have been

identified by phytochemical analysis of the commercially available *S. rhamnoides*. It is essential to conduct future phytochemical studies based on its traditional usage.



Fig. 1. *Sea buckthorn (Hippophae rhamnoides L.)*

Due to dosage selection issues and inadequate controls, a more in-depth research is required. Another research confirming this finding found that polar and non-polar portions of the plant exhibited solid antibacterial capability regardless of the tested plant part (frozen crushed sea buckthorn berries). In dermatology, the *H. rhamnoides* plant is a great help. The presence of polyphenols and fatty acids in plant oil may be responsible for the miraculous benefits of the plant on the skin. *H. rhamnoides* is a superior UV and radio-protectant to sunscreen lotions, as shown by its use in several cosmeceuticals and its traditional medical applications. Sea buckthorn's chemical composition and medicinal properties are also described in detail (Pundir et al., 2021).

Hippophae rhamnoides is a thorny shrub or small tree that is endemic to Eurasia and is a member of the Elaeagnaceae family. The plant's fruit, leaves, and other components are. The plant has been studied intensively for many decades due to its potential health benefits and pharmaceutical uses. Phytochemical studies of SB made considerable strides from 2010 to 2021. Scientists have extracted dozens of novel chemicals from this plant, most of them phenolics. Another fascinating class of phytochemicals found in sea buckthorn is called flavonolignans. One of the most prominent ellagitannins in SB leaves, casuarinin, is the compound that shows the most significant promise. Tests indicated that compounds 43 and 46 had a substantial neuroprotective effect, whereas compounds 47 and 48 exhibited many immunosuppressive qualities. The presence of bioactive chemicals, including triterpenoids, saponins, and ellagitannins, in sea buckthorn likely accounts for most of the plant's pharmacological significance (Żuchowski, 2022).

Due to its high concentration of beneficial compounds, seabuckthorn is a hardy plant. Due to its widespread application in traditional dermatology practices, further in-depth phytochemical studies grounded on actual usage by the traditional population are required. In the realm of healing, homeopathy has many clouts. There is much good stuff in this wonder fruit, but the anti-inflammatory properties stand out. In this analysis, we look at how *Hippophae rhamnoides* are being used to treat various diseases. Both conventional and alternative medicine practitioners have used this plant to treat many conditions (Ahani & Attaran, 2022).

1.1 Medicinal Effects of Phytochemicals

A large body of in vitro and in vivo research suggests the possible therapeutic value of phenolic compounds. The atherosclerotic plaque development process begins with LDL oxidation. Some research has linked dietary phenolics' AO effects to reduced risk of cardiovascular disease. As a result of their influence on nitric oxide synthase, flavonoids are beneficial to cardiovascular health. Bilberry (*Vaccinium myrtillus*) juice anthocyanins have been shown to protect the vascular system in vitro by increasing capillary blood vessel permeability.

Green tea phenolic compound EGCG has been shown to inhibit the growth of chemically generated cancers in experimental animals' esophagus, liver, lungs, skin, and stomach. Ellagic acid is a potent

antioxidant inhibiting peroxy radical and hydroxyl radical-induced DNA damage. Resveratrol has been shown to have anticancer benefits against intestine, colon, and skin cancers in vivo investigations. Epidemiological studies have indicated a link between phenolic chemicals and better health. It has been hypothesized that ingested phenolic AOs from food, medicine, and supplements seldom accumulate therapeutically valuable quantities in the body.

The authors hypothesized that the AOs' free radical scavenging action is at a bare minimum in the 50–100 M concentration range. It has been demonstrated in vitro that xanthine oxidase and nitric oxide synthase, two enzymes involved in the production of free radicals, are inhibited by phenolic AO. Intestinal glucose absorption by rat intestinal epithelial cells has also been shown to be blocked by phenolic chemicals found in tea. Some research has shown that derivatives of caffeic and ferulic acids may prevent skin damage caused by exposure to sunlight. There is evidence that quercetin may inhibit the in vitro replication of herpes simplex virus type I. It is worth noting that phenolic AOs may operate as pro-oxidants by encouraging the production of free radicals under certain situations.

1.2 Phytochemicals in Health Care

Metabolizing and interconverting various chemical substances is essential for all life forms. Despite their vast differences, all life forms have similar metabolic pathways for modifying and generating carbs, proteins, lipids, and nucleic acids. Compounds whose natural occurrence is restricted to a single species or cellular structure are said to be secondary metabolites, and the metabolic process that produces them is known as secondary metabolism. Secondary metabolism produces the most biologically active natural products, including phenolic compounds, alkaloids, and terpenoids.

1.2.1 Lipids and their derivatives

Generally speaking, lipids refer to biomolecules that are insoluble in water but soluble in non-polar solvents. Though this category includes the vast majority of chemical substances, many traditional fatty acids are very soluble in water, highlighting the classification's limits. Lipids may be more narrowly defined as fatty acids and their derivatives, with other hydrocarbon-based natural compounds being handled in a distinct category.

1.2.1.1 Hydrocarbons

Some minor polar natural compounds are hydrocarbons, a small but significant subset. Plants produce aliphatic hydrocarbons by decarboxylating fatty acids; these hydrocarbons often contain an odd number of carbon atoms. Ethylene, the simplest unsaturated hydrocarbon, is a crucial plant hormone that triggers fruit abscission and ripening. In addition to smaller unsaturated hydrocarbons, plants can produce larger ones. Polyacetylenes are a particular class of plant hydrocarbons with several acetylene bonds in their chemical structure.

1.2.1.2 Fatty acids (FAs)

Carbon atom counts in naturally occurring FAs may range from 4 to 30 or even more. Most FAs exist as esters with glycerol, which are referred to as fats or oils. Mixed triglycerides make up the bulk of the majority of naturally occurring fats and oils. The intimate interaction of molecules is reduced when FAs in plants are isomerized selectively into the cis form.

1.2.1.3 Steroids

Steroids are tetracyclic ring-structured modified triterpenoids, namely lanosterol. Cholesterol is an excellent example of the basic framework of steroids. A wide variety of physiologically significant natural compounds may be generated by modifying the side chain. Bioactive substances belonging to the steroid family include sterols, steroidal saponins, active cardiac glycosides, bile acids, corticosteroids, and mammalian sex hormones. Natural and synthetic/semi-synthetic steroidal substances are used frequently in clinical practice.

1.2.2 Aromatics

A large percentage of naturally occurring compounds are aromatic compounds. Aromatic molecules have a role in various plant physiological processes, including pigmentation, photosynthesis, microbial repulsion, and structural organization. Phenols constitute a substantial proportion of all aromatic chemicals. These compounds are produced by biosynthetic routes such as terpenoids, polyketides, and shikimates.

1.2.2.1 Phenolic Compounds

The word "phenolics" in chemistry refers to a broad class of compounds with a wide range of structures and functions. Hydroxylated aromatic compounds are those in which one or more hydroxyl groups are directly linked to an aromatic ring. The phenols are somewhat acidic because the hydrogen is easily lost from the aromatic ring. Harborne and Simmonds categorized these compounds according to the number of carbons present in the molecule. The hydroxyl group of phenols is only mildly acidic because the hydrogen is easily lost from the aromatic ring that contains the hydroxyl group.

(i) *Simple phenolics*

Substituted phenols are referred to as simple phenolics. Common examples of simple phenolics include things, among other things.

(ii) *Phenolic acids*

An abundant class of phenolic chemicals in plants, phenolic acids are essential to plant metabolism. Derivatives of hydroxycinnamic acid and hydroxybenzoic acid make up the bulk of phenolic acids. In nature, phenolic acids may be found in their free and bound states. Many different pharmacological effects, such as antioxidant, anti-mutagenic, anti-tumor, and anti-carcinogenic, have been attributed to them.

(iii) *Flavonoids*

Flavonoids, which are C₁₅ chemicals, all contain the structure C₆-C₃-C₆. Depending on their overall composition, flavonoids may be divided into many categories.

The heterocycle of flavonoids has six members. Three malonyl CoA molecules condense to form the A-ring, whereas one molecule of p-coumaroyl-CoA gives rise to the B-ring. Common flavonoids have a six-member heterocycle, often a pyran, pyrylium, or pyrone ring, to which oxygen is supplied by Common flavonoids have oxygen added to their six-member heterocycle by one of the A-m-hydroxyl ring's groups.

(iv) *Lignans*

The monolignols p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol form the dimers and oligomers known as lignans. Because they are essential in keeping insects at bay, they are concentrated in the plant's woody stems and seeds. Dimers of monolignols connected by an 8-8' (-') bond are commonly referred to as lignan, whereas dimers and oligomers that include linkages other than the 8-8' bond and are referred to as neolignan.

1.3 Physiological effects of SB berries

SB berries and berry products have been linked to various health benefits, including antioxidant (AO) effects, radioprotection, anti-tumor activity, immunomodulatory, cytoprotective, and ulcer- and eczema-preventive properties. SB berries and berry products have been linked to a variety of health benefits, such as antioxidant (AO) properties, radioprotection, anti-tumor activity, suppression of LDL cholesterol oxidation and platelet aggregation, immunomodulation and cytoprotective effects, protection against stomach ulcer, attenuation of atopic dermatitis, and accelerated wound healing. Gao et al. looked at how SB fruit AO activity changes when they ripen. Oatmeal porridge with SB flavonols does not significantly alter oxidized low-density lipoprotein, C-reactive protein, homocysteine, or plasma oxidant potential in people. Cytoprotective and immunomodulatory effects of SB leaf extracts against chromium-induced cytotoxicity.

1.4 Processing of SB berries

Hundreds of companies in China and Russia have developed SB berry-based cosmetics and dietary supplements. Beveridge et al. and Zeb et al. (2004) conducted in-depth reviews of the several methods for preparing SB berries and their subsequent uses. The primary SB berry exports are juice, pulp oil, seed oil, cream, and colors. Although solvent extraction has been explored for oil recovery, it is not advised for nutraceutical applications since the bioactive phytochemicals are destroyed during desolventization. Derevich et al. built an SC-CO₂ extractor for organic oil from SB seeds.

2. Materials and Methods

The study used berries from three distinct SB species, all of which were sourced from important SB growing sites in India's Trans-Himalayan region. Fresh berries belonging to the *H. rhamnoides* var. *turkestanica* species were gathered in the Indus valley, which is located in the Ladakh, Spiti, and Lahaul areas. Berries were harvested in triplicate from three distinct places within the same geographic region, making up three different locations. After removing frozen berries from the freezer, they were smashed carefully so the seeds would not get damaged. After that, the seeds were manually removed from the flesh of the berries.

2.1 Chemicals and Reagents

The following biochemical standards were purchased from Sigma-Aldrich: beta-carotene, fatty acids, -sitosterol, stigmasterol, campesterol, quercetin, kaempferol; ascorbic acid; malic acid; citric acid; gallic acid; protocatechuic acid; para hydroxybenzoic acid; vanillic acid; salicylic acid; cinnamic acid; p-coumaric acid; for (St. Louis, MO, USA). Calbiochem was the vendor for acquiring tocopherols and tocotrienols (Merck Ltd., Mumbai, India). Merck Ltd supplied the Folin-(F-C) Ciocalteau's reagent and HPLC grade hexane, isopropyl alcohol, methanol, water, acetic acid, and formic acid (Mumbai, India). The other chemicals and reagents maintained the analytical grade. Spectroscopic analyses described Isorhamnetin and quinic acid after being separated from SB berries and employed in the experiment.

2.2 Proximate Composition of Berries

Moisture (No. 930.15), protein (No. 988.01), and ash content (No. 942.02) were all measured according to AOAC protocols. Oven drying was used as the moisture test. Soxhlet and Anthrone were used to calculate total fat and sugar content, respectively. The analysis was repeated three times to get a reliable mean and standard deviation.

2.3 Extraction of Lipids-Christies Method

First, we took 1 gram of berries and homogenized them in a 10% potassium chloride solution in water. Then, we shook the mixture vigorously and let it settle. After centrifuging and filtering the mixture, we washed the residue with a combination of chloroform and methanol (1:1, v/v, 30 mL). After removing the solvent using a rotary film evaporator, the lipid layer was filtered and dried over anhydrous Na₂SO₄. We have frozen the lipids at 20 degrees Celsius for future study.

2.4 Total Phenolics

Utilizing a Calibration plot, we determined the total phenolic content (GAE) per gallic acid extract/sample per gram. Extraction yielded 1 gram of extract, which was then filtered, desolventizing, and brought to a final volume of 25 milliliters using methanol. Extracts were oxidized using 0.5 mL of F-C reagent, and the reaction was stopped using 1 mL of 20% sodium carbonate (Na₂CO₃). After 90 minutes of room temperature incubation, the resultant blue solution was analyzed by spectrophotometry at 760 nm to determine its absorbance.

2.4.1 Extraction of flavonoids

Under a nitrogen environment at room temperature, 10 g of berry parts were homogenized with ethanol (6 x 50 mL) and 250 mg of ascorbic acid. We centrifuged the juice at 1500 g to remove impurities, and the remaining clear liquid was analyzed.

2.4.2 Total flavonoids

The aluminum chloride technique proposed by Chang et al. was used to calculate the concentration of flavonols. Using a Shimadzu UV-160A spectrophotometer, we determined that the absorbance of the reaction mixture was 415 nm (Kyoto, Japan). Ten milligrams of quercetin were diluted to 25, 50, and 100 g/mL concentrations by dissolving it in 80% ethanol. 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 1M potassium acetate, and 2.8 mL of distilled water were used to make up the standard solutions.

3. Results and Discussion

3.1 Chemical Composition of SB berries

Amino acids, carbs, organic acids, protein, vitamins, and phenolic substances are plentiful in SB berries. The carbohydrate composition is anything from 8% to 14%, with glucose and fructose making

up the bulk of the carbs in about equal proportions. On a fresh weight basis, the fruit has a protein level that ranges from 0.79 to 3.11 percent. Although reports of vitamin C content in SB vary widely, it is generally regarded as a good source. Yang et al. looked at berries' oil content and fatty acid composition from two SB (*H. rhamnoides* L.) subspecies. Ozerinina and colleagues looked at the makeup and structure of the TAGs in SB seeds of Russian cultivars. *rhamnoides*. The SB berry mesocarp oil from the Caucasian climate type is mainly composed of SU2 and SU3 TAGs, which are high in the isomers C16:0 and C18:1. Carotenoids in SB fruit vary from 30 to 40 mg per 100 g, depending on the local climate. Supercritical carbon dioxide (SC-CO₂), hexane, and cold pressing have all been shown to extract phytosterols from SB (*H.rhamnoides* L.) seed oil. Where you live, you could find carotenoid levels in SB fruit anywhere from 30 to 40 milligrams per 100 grams, with beta-carotene making up about 45 percent of the total.

Characteristics (%)	Pulp	seeds
Moisture	81.3 ± 1.6	64.0 ± 0.9
Fatty Matter	4.5 ± 0.4	7.2 ± 0.6
Protein	2.0 ± 0.2	2.3 ± 0.4
Sugar	8.5 ± 0.7	18.2 ± 1.1
Minerals	1.0 ± 0.2	3.1 ± 0.2
Crude Fiber	1.2 ± 0.3	0.7 ± 0.2

Table.1 Proximate composition of *H. rhamnoides* berry pulp and seed (on fresh wt., Mean % ± SD)

3.2 Vitamin C Content in the Berries

Ascorbic acid (vitamin C) is considered an essential nutrient. Ascorbic acid may either restore the AO activities of other AOs, such as -tocopherol, or operate as an AO by interacting with aqueous radicals. Since vitamin C content is a crucial indicator of juice quality, it is no surprise that SB berries have earned a stellar reputation. For the first time, optimum RP-HPLC-DAD analytical conditions were determined for profiling organic acids in SB berries. Given the polar nature of the organic acids, an RP C18 column was used as the stationary phase. The mobile phase was a 2.4 pH solution of phosphoric acid. During a chromatographic run, UV-visible spectra of analytes may be recorded with the use of DAD.

Composition	Amount
Vitamin C (mg/kg)	0.22 ± 0.02
Organic acids (%)	
Quinic acid	2.80 ± 0.12
Malic acid	1.60 ± 0.09
Citric acid	0.16 ± 0.03
Total organic acids	4.56 ± 0.12

Table.2 Measured as a mean % SD, the vitamin C and organic acid content of *H. rhamnoides* fresh berries (80% moisture) is shown below.

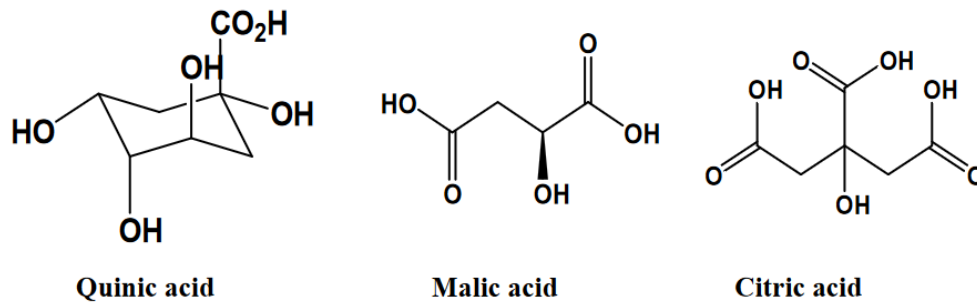
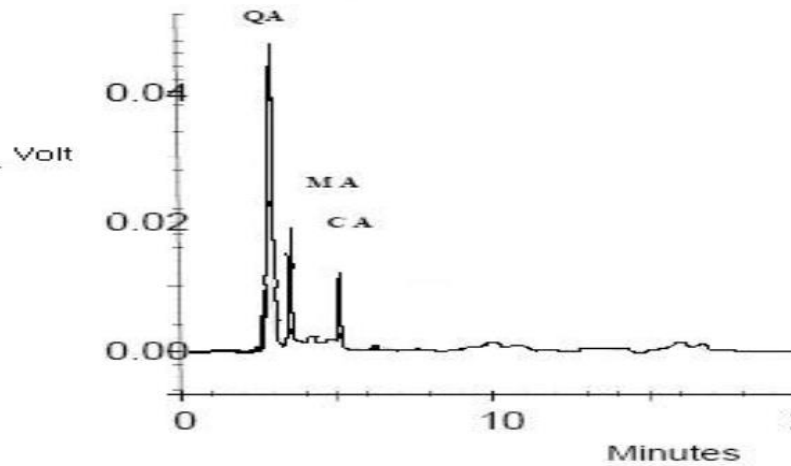


Fig.2 Structures of primary organic acids in SB berries



QA: quinic acid, MA; malic acid, CA: Citric acid

Fig.3 Organic acid HPLC chromatogram in SB berry pulp.

3.3 Polyphenols

Polyphenols are a kind of secondary metabolite found in almost every plant. Natural polyphenols may be any size, from single molecules (phenolic acids, phenylpropanoids, flavonoids) to highly polymerized compounds (lignans, melanins, tannins). These chemicals aid development and reproduction, defend plants against disease and contribute to their aesthetic qualities, such as coloration and smell. Anti-allergic, antibacterial, anti-inflammatory, anti-atherogenic, anti-carcinogenic, and cardioprotective is some of the polyphenols' biological actions. The phenolic content of the pulp was 2.73% by dry weight. Three-point nine five percent and eight-point eight eight eight percent phenolics were found in the seed coat and kernel, respectively. From these findings, we were able to calculate the total phenolic content of the berries and seeds. Whole berries had 3.62% phenolics, whereas seeds had 7.15%. The phenolic content measurements are consistent with those made on Chinese and Polish berries.

	Polyphenols (%)	Flavonols (%)	Proanthocyanidins (%)
Pulp	2.73 ± 0.12	0.21 ± 0.05	1.26 ± 0.14
Seeds Coat	3.95 ± 0.04	0.02 ± 0.00	0.87 ± 0.10
Kernel	8.58 ± 0.32	0.53 ± 0.01	4.40 ± 0.22
Berries	3.62	0.24	1.67
Seeds	7.15	0.35	3.30

Table.3 Total polyphenols, flavonols, and PAs in *H. rhamnoides* berries (dry mass). (Mean ± SD (n = 5)).

3.4 Flavonols

Alu. chlor. A reagent was used to determine the total quantity of flavonols, a flavonoid found in abundance in SB fruit and foliage. The SB berry pulp, seed coat, and kernel were determined to contain 0.21%, 0.02%, and 0.53%, respectively. The plant-derived glycosides and esters of flavonoids are the most common forms of these compounds. The mass spectra revealed a molecular weight of 316 and a fragmentation pattern indicative of flavonols. The flavonoids in the SB berries were resolved using an RP C-18 column and an isocratic methanol-water solution. Reference samples of quercetin, isorhamnetin, and kaempferol were used to improve the RP-HPLC-DAD settings. Isorhamnaethnetin was responsible for 72-80% of flavonols in the SB berry's pulp and seeds.

	Pulp	Seed Coat	Kernel
Quercetin	54.5 ± 8	4.2 ± 3	97.7 ± 11
Kaempherol	19.0 ± 2	0.8 ± 1	14.0 ± 8
Isorhamnetin	214.1 ± 8	13.4 ± 4	441.2 ± 7
Total	287.6 ± 17	18.4 ± 5	552.9 ± 13

Table.4 Flavonoids in *H. rhamnoides* berries (mg/100g of dry weight).

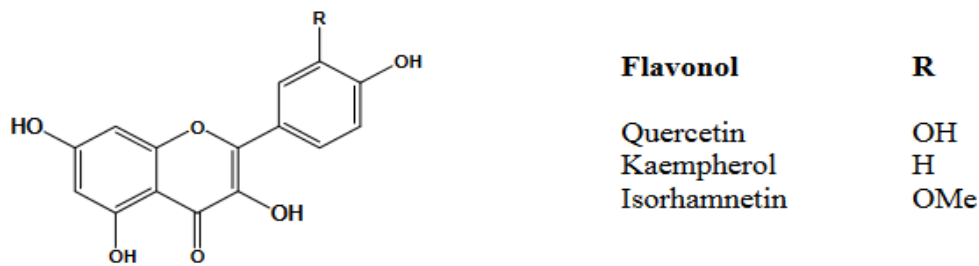
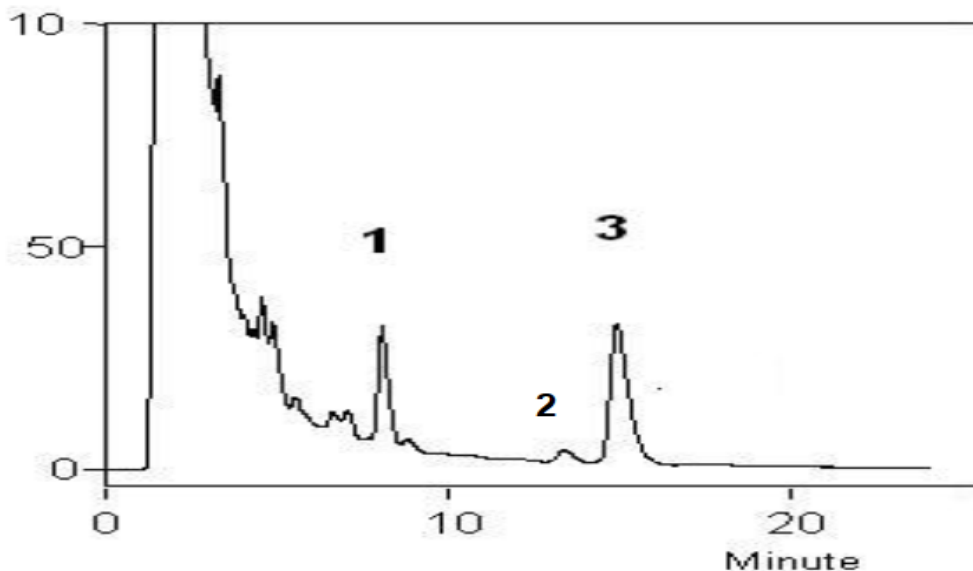


Fig.4 Structures of major flavonols in SB berries.



1: quercetin, 2: Kaempherol, 3: Isorhamnetin

Fig.5 Flavonol chromatogram at 270 nm from *H. rhamnoides* berry pulp HPLC-DAD.

Conclusion

Among the many nutrients included in SB berries are Glucose and fructose comprise the bulk of the carbs, which sit from 8.0 to 14.0%. Fruits have a low (0.79%) to high (3.11%) protein content.

According to popular belief, SB is loaded with vitamin C. Since vitamin C content is a crucial indicator of juice quality, it is no surprise that SB berries have earned a stellar reputation. Ascorbic acid may restore the AO activities of other AOs or function as an AO itself by interacting with aqueous radicals. Alu. Chlor. A reagent was used to determine the total quantity of flavonols, a flavonoid found in abundance in SB fruit and foliage. Finding the optimal conditions for organic acid profiling in SB berries using RP-HPLC-DAD analysis is a first. Three point six two percent phenolics were found in the whole berries, whereas seven point fifteen five percent were found in the seeds.

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