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Studies on the Phytochemistry, Antimicrobial and Antioxidant Properties of Hippophae Rhamnoides

Aman Khajuria[1]*, Dr. Arti Chaurasia[2]

[1]Research Scholar, Deparment Of Chemistry National Institute Of Medical Sciences And Research (NIMS) University, Jaipur

[2]Asst. Professor, Department Of Chemistry NIMS University, Jaipur

*Corresponding Author Email Id:- aman.khajuria458@gmail.com

Abstract

Antibacterial and antioxidant chemicals may be found in H. rhamnoides L., which was a source of these compounds. The phytochemical constituents, antioxidants and antimicrobial characteristics of the plant parts of Himalayan sea buckthorn varieties were studied. There were notable variations in total polyphenols and flavonoids levels amongst the cultivars. The quantity of polyphenolic chemicals in leaves differed more than in berries, according to HPLC analysis. The presence of phenolic chemicals in sea buckthorn leaves and berries, this study found that quercetin as well as hydrocinnamic acid compounds, in particular, are beneficial. Berries include five types of carotenoid pigments, including lutein, zeaxanthin, cis- and beta-carotene. SF6 > Golden Abundant > Carmen > Colosal are the kinds with the most polyphenols, flavonoids, as well as antioxidant activity in their berries, whereas the ranking sequence for leaf extricate is SF6 > Golden Abundant > Colosal > Carmen. There was a substantial connection (r = 0.96), between total flavonoid production and antioxidant activity. S. aureus, B. cereus, and P. aeruginosa were all killed by the extracts, Furthermore, berry extractions were less efficient than leaf extractions.

Keywords: antimicrobial activity; sea buckthorn; carotenoid; flavonoid; antioxidant activity; phenolic compound.

Introduction

Because people are increasingly worried about the safety of artificial substances in handy food items, there has been a rising Antioxidant compounds found in foods as well as green leafy vegetables are becoming more popular across the globe. The cheap, the wide range of synthetic families and quantities of antioxidants found in many plants, the increased bioavailability of antioxidants in the human organism, and the lack of detrimental reactions in the human when compared to synthetic antioxidants are the primary reasons for using vegetable origin as antioxidants and antimicrobials.

The spiny, nitrogen-fixing evergreen shrubs Hippophae rhamnoides L. ssp. carpatica of family Elaeagnaceae is indigenous to Europe and Asia. Sea buckthorn has around 150 cultivars, although new variations are continually being created (Teleszko et al., 2015). Sea buckthorn berries as well as leaves are abundant in bioactive compounds including isoflavones as well as flavonoids, that have a range of health advantages including such anti-atherogenic, antioxidant, anticancer, as well as antibacterial activity (Suomela, et al., 2006). Example of hydrophilic as well as lipophilic antioxidative compounds include ascorbic acid, flavonoid, proanthocyanidins, as well as carotenoids (Fan et al., 2007) (Gao et al., 2000). Sea buckthor leaf, particularly demonstrated to contain more phenolic substances and antioxidant activity than the berries, as well as The more nutrients as well as bioactive components, including such micronutrients, essential fats, carotenoids, as well as phenolic compounds, that are present. Protecting the organism from free radical damage is the primary function of antioxidants. which are formed as a result of metabolic reactions. They also help to prevent pathogenic processes like cancer and cardiovascular disease, as well as improve immunological function. In addition to their antioxidant properties, phenolic chemicals found in SB leaves have been shown to possess antibacterial action in opposition to a variety of pathogen. Furthermore, antibacterial, antitumoral, anti-inflammatory, and antioxidative SBT leaf extracts' characteristics have been demonstrated (Jain et al.,2008) (Geetha et al., 2002) (Ganju et al., 2005).

Varietal, period of harvest, leaves location on the plant, and methodological approaches are all studied to have a considerable impact on phenolic compound concentration and composition. The bioactive components (phenolic, flavonoid, and carotenoid chemicals) in sea buckthorn berries and leaf extracts have been linked to antioxidative and antibacterial activities (Yang et al., 2015) (Vagiri et al.,2013). Although several research on sea buckthorn have been conducted in various parts of the globe, information on the amounts of biologically active substances as well as Research on the antioxidant and antimicrobial properties of Romanian cultivars is very limited. Researchers set out to determine the bioactive chemicals present in four distinct cultivars of Hippophae rhamnoides L. ssp. in terms of determining each one's potential as a bioactive agent. Carpatica's purpose was to provide information that would allow cultivars having high activity to be selected for future growth. One potential future application for the collected data is the development of sea buckthorn-based nutraceuticals for humans and animals. Even though sea buckthorn juice as well as oil extraction produces a large quantity of

"waste" material including leaves, fruit, pulp, as well as seed fragments, these materials contain considerable chemical components that might be turned into a useful animal feed ingredient.

Literature review

(Padmavathi et al., 2005) discovered that they inhibited the development of cutaneous papillomas in mice after exposure to dimethylobenzenoantracen. Glutathione peroxidase, catalase, superoxide dismutation, as well as the glutathione reductase enzymes in the mouse liver may be responsible for the inhibition of carcinogenesis, according to the authors. IRF-1, a transcription factors that acts a key role in cancer cell growth inhibition & death, may be boosted by sea buckthorn fruit's DNA-binding activity, according to new study.

(Wang et al., 2015) antitumor effects of sea buckthorn are not limited to phenolic substances or extracts/fractions: One of HRWP-unique A's properties is that it has both anticancer and immunostimulant properties in the human body, as shown by its ability to inhibit tumour performance and increasing the immune system. HRWP-A was shown to effectively reduce the development of Lewis lung carcinoma (LLC) in mice with tumors in a study of anticancer effectiveness. In tumor-bearing rats, this medicine increased the proliferation of lymphocytes, the activity of macrophages, and the activity of natural killer cells. Polysaccharides dosages of 50, 100, as well as the researchers gave 200 mg/kg intragastrically every day for 14 days.

Aims and objectives

The goal of this research is to look at the phytochemistry, antimicrobial effects of Hippophae rhamnoides L.

To achieve the research's goal, the current study has been divided into the following objectives:

• Making use of standard methods this research will include the phytochemical study of several solvent extracts of medicinal plants that have been selected.

To determine the various effects or antimicrobial properties of SBT plant extracts

Materials and methods

Plant Material

It was possible to get SBB as well as leaves from four different types of sea buckthorn (SF4), Carmen (SF7), Colossal (SF8), as well as SF6 (three of which have been homologated). Refrigeration was kept at -20°C to keep fresh berries as well as leaves until they were ready to be used.

Chemicals

Sigma-Aldrich Co. and Fluka provided standards for sugar, phenolic, as well as carotenoid compounds, while Merck supplied the rest of the reagents (Darmstadt, Germany). The Merck 0.45m MF-Millipore Membrane Filter was used to filter the samples prior to analysis (Darmstadt, Germany).

Sugars Determination

Extract Preparation

For one hour, the samples of SBT (2.5 g) were homogenised on a multiposition magnetic stirring using ultrapure water after being crushed by a grinded. A 50-mL graduated flask poured upto the top with HPLC-pure methanol as well as ultrapure water was used to transfer the mixture. Vials containing the solution were maintained in the freezer at 4 °C until further analysis using a Millipore syringe filter of 0.45 m.

Sugar Determination Using the HPLC Method

The HPLC technique established by Bonta was modified and utilised to analyse the carbs in sea buckthorn fruit. These indexes were detected using an SLC-10 Prop by Shimadzu Liquid Chromatography. We utilised Altima Amino 100 stainless steel columns with an inner diameter of 4.46 millimetres to separate carbs. The carbohydrate had a particle size of 5 micrometres (Alltech, Nicholasville, KY, USA). This thermostatic heater kept the column's temperature at 30 degrees Celsius. The column had a pressure of 6.3 MPa. The stage of mobility was filtered through a porous membrane (0.45m) at a flowing rate of 1.3 mL/min using acetonitrile/water (75:25 v/v) prior to chromatographic analysis. The amount of fluid injected: 10 ml. In order to produce the calibration curve for every sugar, 0.5–80 mg/mL standard solutions were utilised. Across all sugars, the calibration curves' linear regression coefficient was more than or equal to 0.9982. When determining the concentration of sugars, a comparison was made between the peak areas of the two types of sugars. The findings were given as a g/100 g proportion of sea buckthorn berry for each of the sugars.

Protein and Fat Composition

According to established AOAC (Association of Standard Analytical Chemists) methods (AOAC, 2000), we measured the total protein as well as fat content. Sea buckthorn berries were tested for employing the Kjeldahl technique and the Soxhlet extraction method, respectively, to determine total protein as well as total fat content.

Phenolic Compounds: Total and Individual Content

Extract preparation

Each 1 g specimen of sea buckthorn berries as well as leaves was sonicated for 1 hour after being separated 3 times with 5 ml of 95 % ethyl alcohol (50 percent v/v). The mixture solution was centrifuged for 10 minutes (15,269 g), with the supernatants collected as well as stored at 4 °C until analysis.

Total Phenolic Content:

The Folin–Ciocalteu method was used to assess it [52]. Extracted water was combined with Folin–Ciocalteu solution (0.2 N) and Na2CO3 solution (80 litres) (1 M). At a wavelength of 765 nm, the solution blue's absorbance was measured after 20 minutes. In order to evaluate the findings, a calibration curve for gallic acid

solutionsof 0.025–0.15 mg/mL (R2 = 0.9992) was created. GAE (gallic acid equivalent) is measured in milligrammes per gramme of extract. The experiments were carried out three times.

Flavonoids are a kind of flavonoid that is found in plants.

Quercetin as a reference standard was used to quantify flavonoids using an aluminium chloride colorimetric assay designed to be used on a 96-well microplate reader. 100 litres of water and sodium nitrate solution (NaNO2) were diluted with 25 microliters of suitably diluted material. AlCl3 (AlCl3) was added to the solution after 5 minutes. After 6 minutes, the solution was diluted with 50 litres of filtered water and 50 millilitres of 1 M sodium hydroxide. We used a 510 nm wavelength to determine the mixture's absorbance. For the determination of quercetin, a calibration curve (R2 = 0.9987) was constructed using solutions ranging between 0.025 to 0.2 mg/mL. In milligrammes of flavonoid equivalent (mg Qe) every gramme of extract, the researchers measured their results. There were three sets of exams.

Individual Polyphenolic Compounds

For 1 hour, 1 g of samples are extracted from the mixture of ethanol and water (50 percent w/v). Supernatants were recovered after centrifugation (15,269 g) or 10 minutes, microfiltered, and utilised for HPLC/DAD analysis.

Using a Shimadzu Nexera-I HPLC system, an acidic solution water–acetonitrile gradient is employed to assess extracts, as described below. Acetonitrile and formic acid (A) were utilised as solvents to modify pH to 2.5. (B). Starting with 80 percent A, the gradient was reduced to 60 percent over the following 5 minutes, 40 percent at 10 minutes, 20 percent at 15 minutes, and 20 percent over the next 5 minutes. The concentration of solvent (A) was reduced to 10% and held for 5 minutes, then raised to 20% during the following 5 minutes, 80 percent of participants remained at the end of the 40-minute experiment. This range of 220–400 nm was used for all spectral data collection. We employed a variety of compounds to evaluate the detector's linearity: Citrus flavonoids such as Gallic acid, chlorogenic acid, caffeineic acid and Herniarin, as well as flavonoids like quercetin-3-galactoside (Q3G), myricetin (MR), and quercetin (QC), and flavonoids like vitexin (VX) (KA).

Carotenoid Composition Determination

Extract Preparation

With minor adjustments, the extraction process reported by (Breithaupt et al., 2001) was employed. Methayl Ether:Ethyl Acetate: Petroleum ether (1:1:1, v/v/v) was used to extract the sea buckthorn berries' total carotenoids (1 g). In the dark for four hours, the extractions were carried out. In order to separate the mixed extracts from each other, water, diethyl ether, and a saturated NaCl solution were utilised. A rotary evaporator was used to collect the organic phase, which was subsequently evaporated. For HPLC–PDA analysis, A specified amount of ethyl acetate was used to dissolve the extract. All extractions were performed three times.

RP–PAD–HPLC Carotenoid Quantification

The Shimadzu HPLC system was used to analyse carotenoid pigments utilising an LC-20AT solvent delivery module and an SPD-M20A UV–Vis photodiode array detector (PAD). This column (diameter: 24 cm; particle size: 5 m) is made of Phenomenex and it was used to infuse samples. All three solvents were utilised in the mobile phase, were also combinations of these three solvents in the proportions of 90:7:3:1 (V/V/V) and 81:15:4.

Activity of Antioxidants

DPPH Scavenging Activity Measurement

It was found that the antioxidative efficiency of SBT extraction, both Berry & leaf forms, was assessed spectrophotometrically by (Velazquez et al., 2003) using a modified version of the (Brand-Williams et al., 1995) approach. An adequate dilution of the extracts, in the amount of 40 L, was combined with 200 L of 0.02 mg/mL DPPH solution to produce the final 40 L solution. After 15 min at RT, the solution's absorbance was measured at 517 nm. Milligrams of Trolox equivalent measure the amount of radical scavenging activity per gram of substance.

Radical Scavenging Activity at ABTS

AbTS*+ radicals may be scavenged and transformed into a transparent substance by the test, which uses 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS*+). The TEAC value is determined by comparison of the degree of decolorization caused by a chemical to the degree of decolorization caused by Trolox. At ambient temperature as well as darkness for 12 hours, ABTS and potassium persulfate solutions were reacting to obtain the radical, which we named the ABTS*+. This solutions were prepared to be dilute, and an absorption of 0.700 0.025 at 734 nm with ethyl alcohol before to use. The final sol. was tested by adding 17 litres of sample to the solution. Six minutes later, the absorbance was tested. In the concentration range of 0.04 to 0.4 mg Trolox, the standard curve was linear. mg/kg of Trolox equivalent was the unit of measurement used to represent the findings.

Antimicrobial Activity

Samples of the SB were used to test alongside both Gram +ve and Gram-ve bacteria B.cereus as well as Pseudomonas aeruginosa, respectively. A 96-well microtiter plate was used to evaluate the minimal inhibitory concentrations (MIC values). There must be an exact match between optical density and turbidity of the liquid nutrition broth in order to create homogenous inoculums 100 microliters liquid broth containing the dilutions of the extract at concentrations ranges between three to 100 mg/mL were introduced into the bacteria A BioTek Synergy 2 multichannel spectrophotometer was used to measure optical density (OD600 nm) every 15 minutes for 24 hours at 37 °C. This experiment was carried out without the addition of seabuckthorn extracts to see what effect it would have on the microorganisms and their growth curves. Ethanoic extraction from Sea Buckthorn were also evaluated in the lab at 50 percent ethanol: water (v/v) to exclude any chance that the extracts'

antibacterial effects were a result of their ethanol composition. A total of 200 litres was used to incubate all of the samples.

Statistical analysis

As a consequence, the mean standard deviation for each parameter was calculated. GraphPad Prism 8 was used to do the statistical analysis. One-way ANOVA as well as the Tukey-test were used to analyse the data. $P<0.05$ has been used as a cutoff point at which statistical validity could be claimed.

Results and discussion

SBT is considered a rich resource of bioactive substances because of its high concentration of different phytochemicals, however the cultivar is an important factor that impacts the matter as well as composition of SB berries as well as leaves. As a result of this, researchers studied the chemical and phytochemical contents of SB berries as well as leaves from the Himalayan species to determine their health benefits.

Berries sugar, protein and fat content

SBT fruits are made up mostly of sugar so because sweetness of the juice is affected by it. In the 3 major species in sea buckthorn, only glucose and fructose have been found (Yang 2009). Ethyl-D-glucopyranoside was eventually identified as a molecule originally labelled "unknown."

The biochemical components discovered in the berries of the four Romanian sea buckthorn varieties investigated for this study are mentioned in Table 1. The main sugars found were fructose and glucose. In comparison to Colosal, Carmen, as well as Golden Abundant, the fructose levels in SF6 were significantly greater (0.19 % 0.12 %, 0.25 %, 0.12 %, and 0.18%, 0.12 %, respectively). The difference between 1.10 percent and 0.13 percent.

In each cultivar, there was a substantial variation in the quantity of glucose and fructose found. Although the glucose levels discovered in Hippophae rhamnoides samples were similar, they were substantially lower than those identified in H. sinensis or H. mongolica by (Yang 2009). The total amount of fructose and glucose in the extracts ranged from 0.6 percent in S. sp. rhamnoides to 24.2 percent in S. sinensis berries, according to (Yang et al., 2011), while s. sinensis had higher glucose concentrations than s. mongolica but lower fructose levels.

Sucrose was discovered only in the Carmen variety (Table 1), and at a very low level,

According to this study, SBT berries are indeed a major resource of protein and fat (Table 1). There were no statistical significance variations in the level of protein in the four SB fruits that were looked at, ranging from 0.72 % in Carmen to 0.86 % in SF 6.

The SBT berry's smooth parts, in addition to the seeds, contain a high oil content (Yang and kallio 2002). Different types and other factors can have an impact on total oil content. The variants in total fat content among

Golden Abundant (4.86), SF6 (4.61 percent), and Colosal (4.21 percent) were statistically negligible in with us samples, while the Carmen type had such a considerably greater accumulation of 5.71 % (Table 1). (Table 1).

Total and Individual Content of Phenolic Compounds

Total Content of Phenolic Compounds

Assessment of total phenol content (TPC), which is mentioned as mg of gallic acid equivalent (GAE)/g using the Folin–Ciocalteu test, was carried out. When phenolic compounds were examined, they were discovered to be abundant in the leaves, rather than the berries, as showed in Table 2, which summarises the research results.

There are substantial variances ($p > 0.05$) when different letters are used in a column.

	Sample	Total Polyphenols (mg GAE/g)	Flavonoids (mg Qe/g)
Berries	Golden Abundant	14.61± 0.41 ^{c}	7.50 ± 0.13 ^{b}
	SF6	18.66 ± 0.13 ^{a}	9.01 ± 0.23 ^{a}
	Carmen	10.93 ± 0.38 ^{b}	7.32 ± 0.11 ^{b}
	Colosal	10.12 ± 0.26 ^{b}	6.57 ± 0.13 ^{c}
Leaves	Golden Abundant	48.12 ± 0.48 ^{a}	33.58 ± 0.46 ^{b}
	SF6	41.60 ± 0.62 ^{b}	36.58 ± 0.18 ^{a}
	Carmen	42.47 ± 0.53 ^{b}	31.53 ± 0.63 ^{b}
	Colosal	42.10 ± 0.54 ^{b}	32.59 ± 0.50 ^{b}

Table 1 Total phenolic content of SBT.

There were statistically significant variations in total phenolic content amongst sea buckthorn cultivars.There were significant variations in total polyphenol content amongst types, While Colosal (10.12 mg GAE/g) and Carmen (10.93 mg GAE/g) have lower concentrations, SF has the greatest (18.66 mg GAE/g).

TPC was highest in Golden Abundant (48.12 mg GAE/g), Carmen (42.47 mg GAE/g), Colosal (42.10 mg GAE/g), as well as SF6 (41.60 mg GAE/g). Carmen, Colosal, and SF6 had the next highest TPC. As shown in Table 2, the TPC concentrations detected in SBT berries by Bittová et al. [18] range from 10.70 to 17.30 (mg GAE/g1). Although it had the least phenolic concentration in the leaf extracts (Table 2), SF6 had the highest

phenolic content in the fruit. According to the results of it and then prior investigations, Golden Abundant's leaves had the maximum concentration of phenolic compounds, which is consistent with this study's findings.

Flavonoids

Colorimetric analysis of the cultivars' total flavonoid content yielded findings ranging from 9.01 mg quercetin (QE)/g FW in SF6 to 6.57 mg quercetin (QE)/g FW in Colosal (Table 2). As expected, the Colosal berries seemed to have the lowest flavonoid concentration, which was also in line with their overall phenol content (SF6 having the greatest total flavonoid concentration). Flavonoids inside the leaf extracts ranged from 36.58 mg QE/g FW (SF6) to 31.53 mg QE/g FW (Carmen) for SF6 leaves, which was significantly higher than the other three kinds of leaves ($p < 0.05$). Leaf extracts had greater flavonoid and polyphenol content than berry extracts, as did the total quantity of polyphenols.

Phenolic and flavonoid content of individuals

The retention duration, UV–VIS spectra, as well as mass spectral analyses of phenolic compounds were analysed using HPLC, which were then referenced to standards and published data. All samples' spectral data were acquired using diode array detection in the 250–360 nm region (DAD).

One of the most complex phenolic fingerprints was found in sea buckthorn leaves, with 14 distinct phenolic chemicals, comprising nine flavonoid, four cinnamic ellagic acid, and gallic acid, being revealed in the samples examined (one compound). There were just eight flavonoids in berries, with only one gallic acid and one cinnamic acid precursor (one chemical) in them (one compound).

Sea buckthorn's polyphenolic components were found to be more diverse in the leaves than the berries, according to an HPLC investigation. While Carmen and SF6 contained more phenolic compounds than Golden Abundant (11 and 9 recognized compounds, respectively), there was less variation in the quantity of phenolic compounds in their leaf extracts. This pattern did not hold true for berry extracts. By far the least quantity of phenols were produced by Colossal leaf extracts, while the highest were produced by berries.

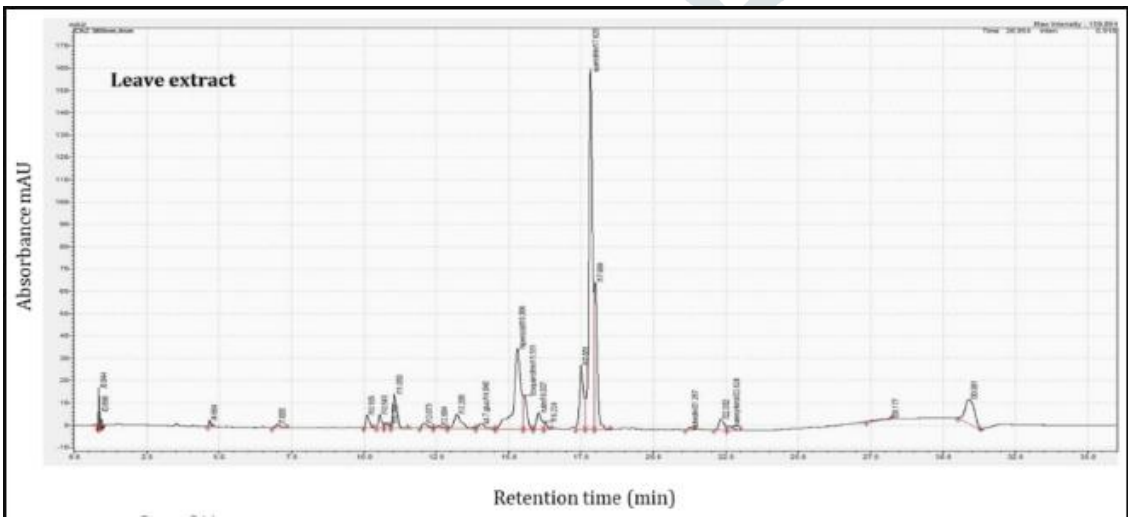

Figure 1Extracts of sea buckthorn leaves were analysed using HPLC.

Flavonol glycoside profiles in berries and leaves may be used to differentiate between distinct SBT taxa.

The greatest overall polyphenolic concentration was found in leaf extracts. The maximum concentration (733 mg/100 g) was discovered in Golden Abundant leaf extracts, while the lowest concentration (152 mg/100 g) was identified in Colosal leaf extracts. There was a high concentration of gallic acid as well as quercetin in the leaves. The caffeic acid content of Golden Abundant's leaves was the highest. while Carmen contained the most vitexin, illustrating the differences in composition.

When it came to chemical concentration, Golden Abundant (Figure 2) seemed to have the greatest level at 19.37 mg/100 g gallic acid, following by SF6 and Colosal (18.58 mg/100 g) (Table 3&4). There were no changes that were statically significant in the gallic acid content of a three strains ($p > 0.05$) except for Carmen's low gallic acid level (6.51 mg/100 g). The most common phenolic acid in sea buckthorn cherries is gallic acid accounting for (Arimboor et al.,2008). Except for gallic acid, the most abundant phenolics in the berries were rutin and quercetin. Only the Carmen samples had vitexin, while the Colosal samples included luteolin-7-glucoside.

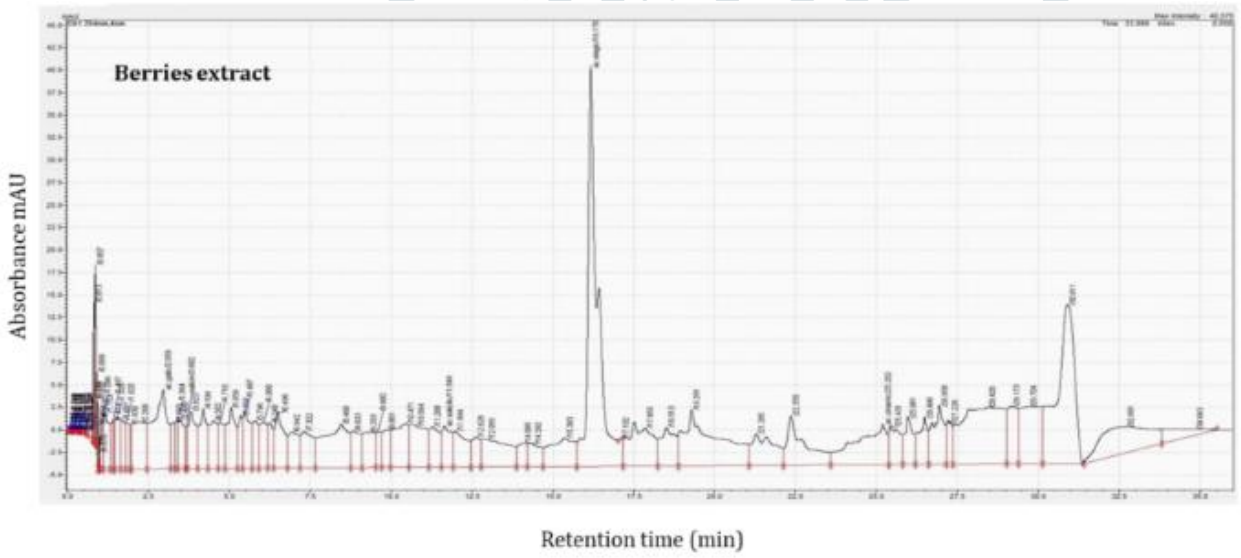

Figure 2HPLC profile of SBT berry extracts.

Compound	Peak	Max Absorption (nm)	RT (min)	GOLDEN ABUNDANT	SF6	CARMEN	COLOSAL
					mg/100 g		
Lutein	1	9.2	421,445,474	1.74 ± 0.04	1.02 ± 0.16	4.74 ± 0.05	0.45 ± 0.07
Zeaxanthin	2	10.3	424,449,476	16.69 ± 0.64	7.62 ± 0.41	27.78 ± 0.55	4.05 ± 0.332
β-Cryptoxanthin	3	38.32	425,449,476	1.05 ± 0.15	0.72 ± 0.19	1.16 ± 0.22	0.16 ± 0.05
cis-β-Carotene	4	58.2	340,444,468	0.36 ± 0.08	0.21 ± 0.16	0.23 ± 0.02	0.80 ± 0.20
β-Carotene	5	61.6	425,450,477	1.94 ± 0.42	0.94 ± 0.36	1.87 ± 0.33	0.17 ± 0.13
Total				**21.78**	**10.51**	**35.78**	**5.63**

Table 2Carotenoid content of saponified sea buckthorn berries.

The total carotenoid yield ranged substantially across types, as found in earlier tests; With the highest concentration of carotenoid SF6 (35.78 mg/100 g) as well as Colosal (56.3 mg/100), Carmen was the most carotenoid-rich of the three. Zeaxanthin and lutein are the major carotenoids inside the Carmen type, that produced more than the SF6 - Colosal variations.

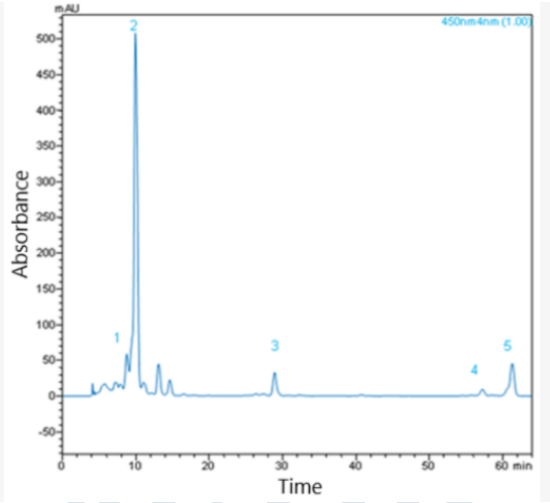

Figure 3HPLC The dissociation of carotenoid in the unsaponified extraction from Carmen variety sea buckthorn berries is shown in this HPLC chromatogram of carotenoids in a saponified profile. Lutein, zeaxanthin, cryptoxanthin, cis-β —carotene, and beta-carotene are the first four peaks, followed by β -cryptoxanthin, and -cis-β —carotene, the fifth.

Zeaxanthin was found in high concentrations in all samples, whereas lutein, β -cryptoxanthin, cis-β —carotene, and -carotene were obtained at much lower concentrations ($p<0.05$). The lutein and -carotene concentrations were identical to the zeaxanthin level. There was a wide range of lutein and -carotene concentrations in Colosal, which ranged from 0.17 to 0.94 milligrammes per 100 grammes, while the quantity of cis—carotene in Colosal ranged from 1.87 to 1.89 milligrammes per 100 grammes.

Carotenoid cis-carotene was found throughout all berry extracts at quantities between 0.21 and 0.80 mg/100g, with no significant variations between kinds ($p >0.05$).

Prior study has shown that carotenoid available from various according on genetic composition, origin, growing conditions, harvest maturity stage, handling and storage, as well as measurement procedures. The significant changes in carotenoid content are compatible with these findings.

Antioxidant Potential

Antioxidant activity of SBT. ssp. carpatica preparations was investigated. Radical cation quench with 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) as well as radical scavenging with 2-diphenyl-1-picrylhydrazyl used to measure the anti-oxidant activity of the extracts as a whole (DPPH).

This action was determined by employing spectrophotometry as well as reported as mg Trolox equivalent per gramme of extracts from sea buckthorn berries as well as leaves (Table 3).

	Sample	**DPPH Method (mg Trolox equivalent/g)**	**TEAC Method (mg Trolox equivalent/g)**
	Golden Abundant	39.25 ± 0.41	32.28 ± 2.35 c
Berries	SF6	42.25 ± 0.23	36.25 ± 3.24 a
	Carmen	36.61 ± 0.52	24.46 ± 2.78 b
	Colosal	39.25 ± 0.35	30.18 ± 2.36 c
	Golden Abundant	138.72 ± 2.76	125.25 ± 3.25 a
leaves	SF6	129.59 ± 2.59	106.28 ± 2.65 b
	Carmen	123.47 ± 1.73	102.28 ± 2.56 b
	Colosal	133.10 ± 3.21	120.58 ± 2.85 a

Table 3 The antioxidant properties of solutions of SBT berries and leaves was determined.

As measured by the DPPH test, each variety's anti-oxidant capacity ranged from 36.61 M Trolox/g FW for berries to 42.25 M Trolox/g for leaf extracts, with SF6 having the maximum antioxidant activity.

Leaf extracts had a stronger antioxidant potential, which was in line with their higher polyphenolic content. After Colosal, SF6, and Carmen, Golden Abundant leaf extract had the greatest scavenging activity with 138.7 mg Trolox equivalent/g of activity, followed by Colosal (133.1) and SF6 (129.6), respectively.

Similar results were seen in French sea buckthorn, which had anti-oxidant activities ranging from 87.0 to 275.0 mg Trolox equivalent/g dry extract as measured by the DPPH test. Sea buckthorn cultivars cultivated in Hungary have anti-oxidant activity (DPPH method) values of 60.37 to 79.10 (mg Trolox equivalent/g extract).

According to the ABTS test, the anti-oxidant capacity of the SBT samples ranged between 130.6 as well as 165.2 mg Trolox/g in the leaves extract, and Golden Abundant proving to be the most effective. Antioxidant activity ranged from 24.46 to 36.25 mg Trolox equivalent/g for the sea buckthorn berry extracts examined in this research, with SF6 having the greatest antioxidant activity of the lot tested. Leaf extracts outperformed berry extracts in the DPPH technique when it came to scavenging free radicals.

In this research, the cultivars scored SF6 > Golden Abundant > Carmen > Colosal in terms of total phenols, total flavonoids content, as well as anti-oxidant activity, as well as SF6 > Golden Abundant > Carmen > Colosal in terms of leaf extract. It was clear that the findings for leaves as well as berries are not consistent. Overall flavonoid endogenous antioxidant activity were shown to have a strong correlation (r = 0.96) in this research. Table 5 shows that the DPPH approach produced somewhat greater antioxidant levels than the ABTS method.

Antimicrobial Properties

Antibacterial activity in opposition to B. cereus, S. aureus, as well as P. aeruginosa was examined in this study using extracts from four distinct varieties of H. rhamnoides L. ssp. carpatica berries and leafs. Next the approach described in the following chapter, we tested each extract's antimicrobial activity by establishing its minimum inhibitory concentration (MIC).

As a means of simulating the obtaining solvent, graphs of production were made using standard growing medium, an ethanol control (100L broth + 100L ethanol 50%), and a standard medium enriched with SBT isolates. This component of the extracts may be discarded since the findings demonstrated that ethanol had no effect on the emergence of the tested bacterial strains at the concentrations used.

To determine the plant extract's minimal inhibitory dose, a series of Dilutions of 3–100 milligrammes/millilitre mediums were conducted. This extract's minimum inhibitory concentration (MIC) was established as the concentration at which bacterial growth was totally inhibited. When MIC extracts were analysed, it was shown that the bacterial strains investigated differed in the amounts of inhibition required (Table 4).

To compare the antibacterial activity (MIC) of Hippophae rhamnoides berries and leaves against four different strains of bacteria, the results are shown in Table 7.

MIC (mg/mL)								
		Berries				**Leaves**		
Microorganism	**Golden Abundant**	**SF6**	**Carmen**	**Colosal**	**Golden Abundant**	**SF6**	**Carmen**	**Colosal**
S. aureus	12.5 ± 1.20	12.5 ± 1.64	25.0 ± 1.86	15.6 ± 1.54	6.20 ± 0.54	6.20 ± 0.54	12.5 ± 1.03	25.0 ± 1.44
B. cereus	25.0 ± 2.35	25.0 ± 1.95	25.0 ± 2.14	31.2 ± 2.32	12.5 ± 0.86	12.5 ± 1.05	12.5 ± 0.92	25.0 ± 1.28
P. aeruginosa	12.5 ± 0.87	12.5 ± 1.54	12.5 ± 0.98	15.6 ± 1.15	6.20 ± 0.68	6.20 ± 0.72	6.20 ± 0.76	12.5 ± 1.06

Table 4 Results of antimicrobial activities against four different bacterial species using Hippophae rhamnoides L. ssp. carpatica berry and leaf extracts (MIC).

Leaf and berry extracts were more efficient than berry extracts in killing S. aureus as well as Bacillus cereus & Psudeomonas aeruginosa. For the extraction, S. aureus showed the greatest sensitivity, whereas Bacillus cereus demonstrated the greatest support. SBT phytoconstituents had significantly lesser antibacterial activity than the MIC towards Staphylococcus aureus. It is possible that this is owing to the large range of sea buckthorn cultivars that exist.

SF6 leaf extract and Golden Abundant have more antibacterial action than Carmen as well as ColosalS. In addition to quercetin derivatives as well as gallic acid, phenolic components found in leaf extracts have a higher antibacterial effect.

Himalayan sea buckthorn leaves were shown to be effective against a variety of medically important bacteria when used at concentrations of 100 to 500/ml. To prevent S. dysenteria at the widest possible zone of inhibition (15.2-mm), Streptococcus pneumonia at the narrowest possible zone of inhibition (2.5-mm) were used instead (8.3 mm). However, the method used to conduct this investigation prevents comparability with the findings of this investigation.

When used in opposition to microorganisms, the antimicrobial activity of French Hippophae rhamnoides L. ethanoic extracts at 100 g/mL changed depending on plants cell nucleus and stress: Streptococus aureus (72%) for extraction from leaves, B.Cereus (64%) for oils from seeds, as well as Enterococcus durans (63% & 68%) seeds extract. Bacterium Pseudomonas aeruginosa was found to be the most resistant to a effects with sea buckthorn extract.

Conclusion

Quercetin derivatives and other bioactive compounds, such as lutolin, gaempferol, etc are all found together in four Romanian SBT cultivars. SBT cultivars' free radical scavenging capacities were shown to be favourably match up with its phenolic and flavonoid content. Polyphenols and flavonoids have been shown to have an antimicrobial impact as concentrations as well as purity have increased. There are a few possible explanations for the antibacterial action of quercetin against the Bacillus cereus as well as Pseudomonas aeruginosa, however the source of quercetin against Staphylococcus aureus remains unknown. There was no evidence that S.aureus relied on any other component of the plant other than vitexin, which was linked to higher levels of quercitrin as well as quercetin-3-galactoside in Carmen cultivar berries, as well as higher levels of Digitoflavone, Luteolin-7-O-glucoside, as well as 4-Hydroxycinnamic acid on the Colosal crop leaf surface. Depending Based on these results, we may inferred that some species of SBT as well as other parts of the herb will be utilized as major sources of antioxidants as well as antimicrobials, but also that new products should be developed to promote their broader use.

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