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Original Research Article

Screening of in-vitro antioxidant activity of *Hippophae salicifolia* berries extract

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Abstract

The present study aimed to study the preliminary phytochemical analysis, standardisation and in-vitro antioxidant evaluation of Hippophae salicifolia (HS) berries extracts. Briefly, the berries were subjected to alcoholic and aqueous extraction. The preliminary phytochemical analysis was performed for aqueous and alcoholic extracts. The aqueous and alcoholic extracts are standardised for total phenolic, total tannin and total flavonoid content. The extracts were evaluated for its in-vitro free radical scavenging activities against, 2'-diphenyl-1- picrylhydrazyl (DPPH) radicals, hydroxyl radicals, nitric oxide radicals and total antioxidant activity. We found that alcoholic extract showed the presence of alkaloids, phenols, tannins, saponins, flavonoids and aqueous extract showed the presence of flavonoids, tannins, carbohydrates, and phenols. The extracts of HS berries were standardized for their total phenolic, tannin, flavonoid and vitamin-C content. The in vitro antioxidant activity revealed that both the extracts showed significant scavenging activities against free radicals. It can be concluded that both the extracts are effective in scavenging the free radicals. The flavonoids, tannins, vitamin-C and saponins may be attributed to free radical quenching activity of Hippophae salicifolia.

Key words

Free radical scavenging, Antioxidant, Hippophae salicifolia, Berries, Extract.

Introduction

Free radicals are produced as a by product during metabolism in human beings. These

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liberated free radicals cause damage to cellular bio-molecules such as lipids, proteins and DNA [1]. Our body defensive mechanism in the form of antioxidants against the free radicals, maintains equilibrium between generated free radicals and antioxidants. If any riot in this equilibrium leads to oxidative stress [2]. It is responsible for a number of pathological diseases such as aging, cancer, diabetes, atherosclerosis and other neurodegenerative disorders [3]. An inequity between free radicals and the innate antioxidant capacity of the body, intended for the use of antioxidants [4]. Natural antioxidants have better therapeutic efficacy in protecting cells from the injury of free radical over synthetic one. So, immense interest is focused on the development of antioxidants from traditional herbal plants.

Hippophae salicifolia of Elaeagnaceae family is one such plant species distributed in Northern Himalayas. The plant is rich in tocopherols, carotenoids, vitamin-C, omega-3 fatty acid and vitamin-K. Tocopherol, vitamin-C, omega-3 fatty acids are shown to have potent antioxidants [5]. Therefore to have the scientific validation on antioxidant activity, the berries were taken for the present study. The aqueous and alcoholic extracts of HS berries were used to investigate the antioxidant activity using in-vitro methods.

Material and Methods

Hippophae salicifolia berries

Hippophae salicifolia berries powder was purchased from Changsha Organic Herb Inc., China. The alcoholic extract was prepared by subjecting the berry powder to extraction with alcohol, refluxed for about 6-8 hours, filtered and evaporated to dryness by rotary film evaporator at low temperature under reduced pressure. The percentage yields were found to be 4.27% w/w. The aqueous extract was prepared by heat distillation method. The percentage yield was found to be 7.63% w/w. The extracts were subjected to various chemical tests in order to detect the presence of different phytoconstituents [6, 7].

Standardization of HS

The total phenolic content of the extract was determined with the Folin-Ciocalteau assay and expressed as milligrams of gallic acid equivalents (GAE) per 100 grams dry mass (mg GAE/100g) [8]. The total flavonoid content was measured with an aluminium chloride colorimetric assay and expressed as milligrams of (+) Quercetin equivalents (QE) per 100 gram dry mass (mg QE/100 g) [9]. The total tannin content was determined by the Folin-Denis assay method and expressed as the equivalents of tannic acid (TE) (mg TE/100g extract) [10].

In vitro antioxidant activity

The free radical scavenging activity of the extracts of Hippophae salicifolia berries was determined by using various in vitro assays such as DPPH radical scavenging assay, hydrogen peroxide radical scavenging assay, nitric oxide radical scavenging assay, total antioxidant capacity, reducing power assay.

DPPH radical scavenging assay

DPPH scavenging activity was measured with Spectrophotometric Method [11]. To the extract solution of concentration ranging from 40 microgram to 200 microgram, 4 ml of DPPH was added and was made up to 5 ml with ethanol, incubated for 30 minutes at room temperature. The absorbance was measured at 517 nm against blank. The percentage of inhibition of DPPH was calculated as follows. Ascorbic acid was used as standard and the scavenging effect of DPPH was expressed in terms of ascorbic acid equivalents.

$$\%\text{ of inhibition} = \frac{\text{Absorbance}_{\text{Standard}} - \text{Absorbance}_{\text{test}}}{\text{Absorbance}_{\text{Standard}}} \times 100$$

Hydrogen peroxide radical scavenging assay

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The scavenging effect of hydrogen peroxide was determined according to the method of Ruch, et al. [12]. 1 ml of extract solution was treated with 0.6 ml of hyrogen peroxide for 10 minutes; the absorbance was read at 230 nm against blank. Ascorbic acid was used as standard and the scavenging effect of hydrogen peroxide was expressed in terms of ascorbic acid equivalents.

Nitric oxide scavenging assay

Nitric oxide was generated from sodium nitroprusside and its scavenging effect was determined [13, 14]. Different concentration of extract solution in phosphate buffer was incubated with sodium nitroprusside for 5 hours at 25 °C. Control experiments were performed with equal amount of buffer instead of extract solution. After 5 hours of incubation, 0.5 ml of supernatant liquid was removed and 0.5 ml of Griess reagent was added. The absorbance of the chromphpore formed during diazotization with sulphanilamide and its subsequent coupling was read at 546 nm. Ascorbic acid was used as standard and the nitric oxide scavenging was expressed in terms of ascorbic acid equivalents.

Total antioxidant capacity

The total antioxidant capacity was determined by Spectrophotometric method [15]. Extract test solution of concentration ranging from 40 to 200 µg was taken in eppendroff tube and 1 ml of reagent containing 0.6 mM sulphuric acid, 28 mM sodium phosphate and 4 mM ammominum molybadate were added. The tubes were incubated at 95 °C for 90 minutes and were cooled to room temperature; the absorbance was read at 695 nm. Ascorbic acid was used as standard and the total antioxidant capacity was expressed in terms of ascorbic acid equivalents.

Results

Preliminary Phytochemical analysis and Standardization of Hippophae salicifolia

The alcoholic and aqueous extracts of Hippophae salicifolia berries were tested for different phytoconsituents like alkaloids, glycosides, saponins, tannins, terpinoids, sugars, phenolic compounds, flavanoids, protein, carbohydrates and volatile oils using standard procedures and the results are given in **Table - 1.** The total phenolic, tannin, and flavonoid content of alcoholic and aqueous extracts of HS were quantified and results were given in **Table - 2.**

Table - 1: Preliminary Phytochemical analysis of alcoholic and aqueous extracts of Hippophae salicifolia berries.

Test for	Alcoholic Extracts of HS	Aqueous Extracts of HS
Alkaloids	-ve	-ve
Tannins	+ve	+ve
Phenolic compounds	+ve	+ve
Glycosides	-ve	-ve
Proteins	-ve	-ve
Flavonoids	+ve	+ve
Sterols	+ve	-ve
Fixed oils	-ve	-ve
Volatile oils	-ve	-ve
Triterpenoids	-ve	+ve
Saponins	-ve	-ve
Sugars	+ve	+ve
Carbohydrates	-ve	+ve

DPPH radical scavenging activity

DPPH radical scavenging activity of alcoholic and aqueous extracts of HS was measured and compared with Ascorbic acid. The percentage inhibition of DPPH radical by the extracts was found to increase with increasing concentration of extracts as well as standard ascorbic acid. The percentage inhibition and IC_{50} concentrations of extracts and ascorbic acid are given in **Table - 3.**

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Table - 2: Standardization of extracts of HS berries.

	Alcoholic Extracts of HS	**Aqueous Extracts of HS**
Total phenols (mg GAE/ 100g extract)	14.36 ± 1.23	65.89 ± 1.11
Total tannins (mg TAE/ 100g extract)	22.78 ± 1.06	23.55 ± 1.57
Total flavonoids (mg QE/ 100g extract)	67.42 ± 1.43	54.68 ± 0.96

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity of alcoholic and aqueous extracts of HS was measured and compared with Ascorbic acid. The percentage inhibition of hydrogen peroxide radical by the extracts was found to increase with increasing concentration of extracts as well as standard ascorbic acid. The percentage inhibition and IC_{50} concentrations of extracts and ascorbic acid are given in **Table - 4.**

Nitric oxide scavenging activity

Nitric oxide scavenging activity of alcoholic and aqueous extracts of HS was measured and compared with Ascorbic acid. The percentage inhibition of nitric oxide by the extracts was found to increase with increasing concentration of extracts as well as standard ascorbic acid. The percentage inhibition and IC_{50} concentrations of extracts and ascorbic acid are given in **Table - 5.**

Total antioxidant activity

Total antioxidant activity of alcoholic and aqueous extracts of HS was measured and compared with Ascorbic acid. The percentage of total antioxidant capacity of the extracts was found to increase with increasing concentration of in extracts as well as standard ascorbic acid. The percentage of inhibition and IC_{50} concentrations of extracts and ascorbic acid are given in **Table - 6.**

Discussion

The Hippophae salicifolia berries were extracted with alcohol and water. The extracts were tested for different phytoconsituents like alkaloids, glycosides, saponinins, tannins, terpinoids, reducing sugars, phenolic compounds, flavanoids, proteins, carbohydrates and volatile oils. The information on the phytochemical composition of a plant will give insight to screen for its biological properties [16, 17, 18]. The extracts are standardized for its total phenolic, tannin and flavonoid content. Now-a-days, plant materials rich in phenolic compounds are used widely in industries to improve the quality and nutrition of the food [19]. Phenolic compounds are considered as secondary metabolites. Flavonoids are recognized as polyphenolic compounds. Phenolics and Flavonoids are entangled as potent free radical scavengers and exert its antioxidant activity due to the presence of hydroxyl group [20, 21]. They are shown to have biological properties such as anti-inflammatory, anti-hepatotoxic, anti-ulcer, anti-allergic, anti-viral and anti-cancer activities [22].

An in vitro antioxidant studies are widely used to screen various plants containing phenolic and flavanoid constituents. Free radicals are known to play a unambiguous role in a wide variety of pathological manifestations. Antioxidants fight against free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or strengthening the antioxidant defense mechanisms. DPPH radical, a free radical widely

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used for evaluating radical scavenging activity of the plant extract [23]. Scavenging of DPPH radical is related to the inhibition of lipid peroxidation [24]. Antioxidants either transfer an electron or a hydrogen atom to DPPH radical, thus neutralizing its free radical character [25]. In the present study, the percentage of scavenging effect on the DPPH radical was concomitantly increased with the increase in the concentration of both the extracts. From the results it is observed that HS berries possess hydrogen donating capabilities, through which scavenges the free radicals.

Hydrogen peroxide, an oxidative metabolite turn to generate hydroxyl radicals (OH) resulting in initiation and propagation of lipid peroxidation. The hydrogen peroxide scavenging activity of alcoholic and aqueous extract of HS was measured and compared with Ascorbic acid. The extracts showed good hydrogen peroxide scavenging activity. The ability of the extracts to quench OH radical seems to be directly related to the prevention of the lipid peroxidation [26].

Peroxy nitrite is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. Nitric oxide (NO) is a diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities [27]. The Nitric oxide scavenging activity of alcoholic and aqueous extract of HS was measured and compared with Ascorbic acid. The total antioxidant capacity of the extracts was measured spectrophotometrically through ammonium molybdate method. The present study demonstrated that both the extracts exhibited highest antioxidant capacity for ammonium molybdate reduction.

Conclusion

It can be concluded that HS berries extracts showed potent anti-oxidant activities as evident from scavenging against different radicals. The aqueous extract showed highest antioxidant activity than alcoholic extract of HS berries. The content of total phenols, total flavonoids may be attributed to its antioxidant activity.

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Conflict of interest: None declared.

Table - 3: DPPH radical scavenging activity of extracts of HS.

Concentration of Ascorbic acid, Alcoholic Extracts of HS and Aqueous Extracts of HS (µg/mL)	Percentage of inhibition		
	Ascorbic acid	**Alcoholic Extracts of HS**	**Aqueous Extracts of HS**
40	90.16 ± 0.91	42.65 ± 0.61	37.44 ± 1.58
80	92.44 ± 1.06	59.66 ± 0.45	49.77 ± 1.37
120	94.17 ± 1.21	63.78 ± 0.37	51.98 ± 1.78
160	95.33 ± 1.54	74.67 ± 1.02	62.67 ± 1.85
200	97.11 ± 1.91	78.12 ± 1.11	72.56 ± 1.99
IC_{50}	**20.36 µg/mL**	**48.22 µg/mL**	**119.34 µg/mL**

Values are expressed as Mean ± SEM (N=3 Readings).

Table - 4: Hydrogen peroxide scavenging activity of extracts of HS.

Concentration of Ascorbic acid, Alcoholic Extracts of HS and Aqueous Extracts of HS (µg/mL)	Percentage of inhibition		
	Ascorbic acid	**Alcoholic Extracts of HS**	**Aqueous Extracts of HS**
40	26.40 ± 3.46	49.59 ± 0.23	64.51 ± 0.22
80	35.91 ± 1.25	61.80 ± 0.32	66.36 ± 0.25
120	42.17 ± 3.59	64.64 ± 0.31	68.92 ± 0.31
160	69.58 ± 2.28	66.01 ± 0.15	73.68 ± 0.28
200	87.23 ± 3.31	76.35 ± 0.28	76.84 ± 0.15
IC_{50}	**142.82 µg/mL**	**40.38 µg/mL**	**30.76 µg/mL**

Values are expressed as Mean ± SEM (N=3 Readings).

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Table - 5: Nitric Oxide scavenging activity of extracts of HS.

Concentration of Ascorbic acid, Alcoholic Extracts of HS and Aqueous Extracts of HS (µg/mL)	**Percentage of inhibition**		
	Ascorbic acid	**Alcoholic Extracts of HS**	**Aqueous Extracts of HS**
40	26.40 ± 3.46	47.42 ± 1.21	45.34 ± 0.74
80	35.91 ± 1.25	48.55 ± 2.01	48.56 ± 0.22
120	42.17 ± 3.59	51.98 ± 0.51	52.98 ± 0.64
160	69.58 ± 2.28	54.22 ± 0.15	58.77 ± 0.01
200	87.23 ± 3.31	64.10 ± 0.01	68.99 ± 0.01
IC_{50}	**142.82 µg/mL**	**119.5 µg/mL**	**115.23 µg/mL**

Values are expressed as Mean ± SEM (N=3 Readings).

Table - 6: Total antioxidant capacity of extracts of HS.

Concentration of Ascorbic acid, Alcoholic Extracts of HS and Aqueous Extracts of HS (µg/mL)	**Percentage of inhibition**		
	Ascorbic acid	**Alcoholic Extracts of HS**	**Aqueous Extracts of HS**
40	57.69 ± 1.27	46.47 ± 0.54	55.94 ± 0.78
80	68.57 ± 2.98	51.93 ± 0.89	62.79 ± 0.84
120	75.75 ± 1.92	62.86 ± 0.78	70.24 ± 0.37
160	85.14 ± 2.91	83.26 ± 0.96	75.58 ± 0.65
200	96.67 ± 1.91	89.97 ± 0.078	87.96 ± 0.66
IC_{50}	**36.21 µg/mL**	**77.67 µg/mL**	**36.38 µg/mL**

Values are expressed as Mean ± SEM (N=3 Readings).

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