ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF WILD BERRIES FROM HIMALAYAN REGION

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Abstract

The aim of the current research is to assess the antioxidant and antimicrobial activities of extracts of five wild berry fruit species from Himalayan Region. Fruit extracts from wild berries might be used to make dietary supplements and pharmaceuticals. It could be concluded from the study that the wild Himalayan berries could be a potential source of rich antioxidant molecules and may be helpful in obtaining new lead molecules for treating several conditions. According to this study, all plant extracts contain interesting antibacterial characteristics that can be related to their abundance in phytochemicals, including phenolics and flavonoids.

Keywords: E. oleraceae, V. myrtillu, P. embilica, R. ellipticus, R. niveus, Berries, Antioxidant, Antimicrobial Activity

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Introduction

High intake of fruits and vegetables is advised by the dietary standards [1-3]. Berries and other fruits are rich in nutrients and phytochemicals that have been shown to support the immune system and prevent a number of physiological problems. In addition to being a rich supply of nutritive components like sugars, essential oils, carotenoids, vitamins, and minerals, berries are also a rich source of non-nutritive, nutritive, and bioactive substances such flavonoids, phenolics, anthocyanins, phenolic acids, stilbenes, and tannins. These vibrant fruits may be frozen, eaten raw, or processed to make a variety of items [4-7]. There are a number of studies on different fruit polyphenols and their antioxidant and antimicrobial activities, some of them include blackberry varieties and no works of *E. oleraceae*, *V. myrtillu*, *P. embilica*, *R. ellipticus* and *R. niveus* berries. Our aim was to evaluate antioxidant and antimicrobial activities of those wild species [8-9]. This study aimed to determine the antioxidant and antimicrobial activity of extracts of *E. oleraceae*, *V. myrtillu*, *P. embilica*, *R. ellipticus* and *R. niveus* berries.

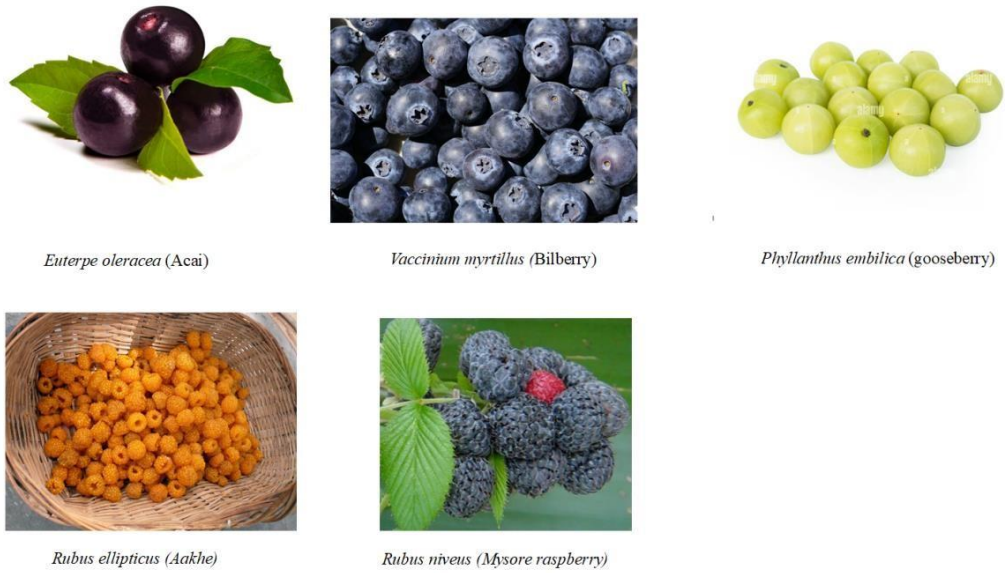

Figure 1: *Ripen fruits prior to their storage at -20 ºC for extracts preparation*

Material and Methods

Fruits and seeds of *E. oleraceae*, *V. myrtillu*, *P. embilica*, *R. ellipticus*, *R. niveus* and Berries Powder procured from the Herbalveda.

Preparation of Plant Extracts

Soxhlet extraction of extracts

The collected fruits and seeds were allowed for surface sterilization using 0.1% mercuric chloride, followed by washing under running water, and left to air dry in the shade for 2 to 4 weeks. The dried fruits and seeds are then finely crushed and kept in an airtight container. The air-dried powder (5 g) was extracted successively by Soxhlet extraction with solvents of different polarity i.e., hexane, petroleum ether, chloroform, methanol, and water. The extraction process employed 450 mL of hexane solvent and continued for 6 to 8 hours (4–6 cycles per hour i.e., 24 to 48 cycles). To monitor the progress of extraction, a sample of the extract was collected from the siphon tube of the extractor every four cycles. The extract was then spotted on a TLC plate and visualized in an iodine chamber to determine the completeness of the extraction. No spot indicates completion of extraction. The dried, sterilized, and airtight extract was collected in a hexane solvent container. A similar different extract was prepared in different solvents. All the dried extracts were weighed. All the extracts were diluted to 20 mL in their respective solvents [10-11].

Characterization of Plant Extracts

Yield

The extraction yield, defined as the ratio of the mass of extract to the mass of dry matter, served as a measure to evaluate the impact of extraction conditions. The percentage of extracted yield was calculated by the formula given below.

Extraction yield/yield% = (WO/Wt) *100

WO = weight of initial fruit/seed sample

Wt = weight of dried extract after Soxhlet extraction [10-11]

Phytochemical Analysis

Using established techniques, the dried powdered sample's qualitative phytochemical properties

were identified and analyzed for the presence of several secondary metabolites. For determining the flavonoid and total phenolic content, the Folin-Ciocalteau reagent method was employed and the aluminum chloride ($AlCl_3$) method, respectively [12-13].

Antioxidant Activity of the Extract

Free Radical Scavenging Activity of DPPH

The DPPH free radical scavenging activity (RSA) was measured after the method developed by **Rawat *et al.*, (2010)** was slightly altered before being put to use. 2010). After waiting 20 minutes in the dark at room temperature, DPPH solution (0.1 mM in methanol, 5 ml) was mixed with various extract dilutions in methanol. This mixing took place in methanol. The control was prepared in the samemanner as it had been in the past, but this time there was no extract included. The absorbance was measured at 517 nm and compared to a blank of methanol. The following equation was utilized in order to arrive at the value for the percentage of radical scavenging activity (RSA):

$$\text{Scavenging activity} = \frac{A_c - A_s}{A_c} X\ 100$$

Both the samples and the control had absorbances that were denoted by the letters A c and A s, respectively. The DPPH free RSA was reported as the amount of catechin equivalent (CE) in milligrams per one hundred grammes of fresh weight [14-17].

Capacity of the superoxide anion to scavenge free radicals

The reduction of NBT was used to measure the superoxide anion radicals produced by the oxidation of NADH in PMS-NADH systems. Tris-HCl buffer (16 mM, pH 8.0, 3.0 ml), NADH (78 mM, 1 ml), and various fruit extract dilutions in water were all part of the reaction mixture. After adding PMS (10 M, 1 ml) to the mixtures, the reactions were started by incubating them for five minutes in the dark at 25°C. The absorbance was lower than that of the water control at 560 nm. Using the same formula as before, the superoxide anion scavenging activity was calculated as mg of ascorbic acid (AAE) per 100g FW [14-17].

Antimicrobial Activity of the Extract

Evaluation of the antimicrobial activities of selected wild edible fruitextracts

Bacterial strains.

All extracts of selected wild edible fruits were tested for antibacterial activity using the disc diffusion assay **(Gupta and Aneja, 2004)** against bacterial cultures of *Escherichia coli (MTCC739), Bacillus subtilis (MTCC441), Staphylococcus aureus (MTCC96), Micrococcus luteus (MTCC106),* IMTECH in Chandigarh provided the bacterial and fungal stains.

Bacterial cultures were grown in nutrient broth (Hi-media Pvt. Ltd., Mumbai, India)and kept at 4°C, while fungal cultures were grown in Sabouraud Dextrose Agar (SDA) (Hi-media Pvt. Ltd., Mumbai, India).

Preparation of inoculums

A single colony of selected bacterial strains was inoculated in 5.0 ml nutrientbroth and incubated for 24 hours at 37ºC in a rotary shaker. A spectrophotometer (600 nm) was used to standardize cell density to approximately 10^8 cells/ml. Culturing the fungal inoculums for 5 days on SDA medium at 28ºC produced the fungal inoculums. 10 ml of distilled water was poured into each Petri dish. Each fungus' spore density was adjusted using a spectrophotometer (595 nm) to achieve a final density of approximately 10^5 spores/ml.

Determination of Antimicrobial Activity

The disc diffusion technique was used to calculate the antibacterial zone of inhibition. After incubating the bacterial and fungal spore suspensions for 24 hours at 45–50ºC, 1 ml was added to 100 ml of sterile nutrition agar medium and SDA agar. After thoroughly mixing the contents of the Petri dishes, they were allowed to cool and harden. The extracts were sterilized using 0.45- µm Millipore filters. After spreading test organisms on plates, 1 ml of undiluted extracts (equal to 66 mg/ml of various fruits) were impregnated onto sterile filter paper discs (Whatmann No # 1, 5 mm in diameter) and incubated at room temperature for 48 hours. Positive controls included streptomycin (10 mcg/disc), and negative controls included the solvents methanol, acid methanol, acetone, and acid acetone. 24 hours of incubation at 37 ºC for bacteria was followed by a visual inspection of the zone of inhibition surrounding each well.

By making dilutions of each fruit extract, the minimum concentrationneeded to stop the growth of microorganisms was also found. To determine the level of inhibition, we used the following formula.

Diameter of inhibition $zone$ by fruit extracts:

$$\%\ inhibition = \frac{Diameter\ of\ inhibition\ zone\ by\ fruit\ extracts}{Diameter\ of\ inhibition\ zone\ by\ standard\ antibiotic} X100\ \%$$

Result & Discussions

Percentage yield of various solvent extracts is shown in table 1.

Table 1: Percentage yield of various solvent extracts

Sample	Final dry weight	Extraction yield (%)
EoH	1.951	39.02
EoP	2.41	48.2
EoC	2.652	53.04
EoM	2.905	58.1
EoW	2.01	40.2
VmH	2.251	45.02
VmP	2.434	48.68
VmC	1.982	39.64
VmM	2.97	59.4
VmW	1.421	28.42
PeH	1.291	25.82
PeP	2.191	43.82
PeC	1.091	21.82
PeM	1.329	26.58
PeW	1.293	25.86
ReH	1.291	25.82
ReP	3.209	64.18
ReC	2.981	59.62
ReM	2.822	56.44
ReW	2.901	58.02
RnH	2.932	58.64
RnP	2.341	46.82
RnC	2.101	42.02
RnM	1.924	38.48
RnW	2.871	57.42

The preliminary phytochemical analysis was done as per reported the literature and results are as shown in table 2.

Table 2: Preliminary phytochemical screening of various solvent extract

Sample	Alkaloid			Flavonoid	Phenol	Glycosides	Tannins	Carbohydrate			Saponins	Steroids
	Mayer's test	Dragondroffs test	Wagner test					Molisch	Fehlings	Benedicts		
EoH	+	+	+	+	+++	-	-	+	-	+	-	-
EoP	+	-	+	+	+	-	-	+	-	+	-	-
EoC	-	-	-	-	-	-	-	+	-	+	-	-
EoM	-	-	-	+++	+++	-	+	++	++	++	-	-
EoW	-	-	-	+++	+++	-	+	++	++	++	-	-
VMH	-	-	-	++	+	-	-	++	++	++	-	-
VmP	-	-	-	+	+	-	-	++	++	++	-	-
VmC	-	-	-	+	+	-	-	++	++	++	+	+
VmM	+	+	+	+++	+++	+	+	+++	+++	+++	+	+
VmW	+	+	+	+++	+++	+	+	+++	+++	+++	+	+
PeH	-	-	-	+	+	++	++	+	-	-	+	-
PeP	-	-	-	+	-	+	+	-	-	-	+	+
PEC	+	-	-	+	-	+	-	-	-	-	+	+
PeM	++	+	+	++	+++	-	++	+++	+++	++	-	-
PeW	-	-	-	-	++	+	++	+++	+++	++	-	-

ReH	+	+	-	+	-	++	-	-	-	-	-	-
ReP	++	++	-	++	+	++	-	-	-	-	-	-
ReC	+	++	+	++	++	++	-	-	-	-	-	-
ReM	++	+++	-	+++	++	++	+++	+	+	++	+	-
ReW	++	+	+	++	++	++	+	++	++	++	+	-
RnH	-	-	-	+	+++	-	-	+	+	+	-	-
RnP	-	-	-	+	++	-	-	+		+	-	-
RnC	-	-	-	+	+++	-	+	+	++	+++	-	-
RnM	+	-	-	++	+++	-	+	++	++	++	+	+
RnW	+	-	-	++	+++	-	+	++	++	++	+	+

Selected fruit extracts were tested for their antioxidant properties.

ABTS scavenging activity

Table 3: ABTS scavenging activity

DPPH free radicals scavenging activity mg CE/100 g FW	Methanol	Acidic- Methanol (pH 2)	Acetone	Acid- Acetone (pH 2)
R. ellipticus	619.6±31.13	704.9±29.74	1072.6±42.11	857.8±38.91
E. oleraceae	557.09±31.8	700.62±25.61	729.45±18.10	446.55±34,52
V. myrtillu	27.59±7.64	32.64±3.28	32.77±5.94	24.1±6.94
P. embilica	493.7±24.58	562.99±23.64	546.73±15.32	666.96±30.95
R. niveus	569±11.9	614.62±27.6	1003.2±36.9	1252±23.7

Each value is expressed as mean ± standard error (n = 3). Each value is expressed as mean ± standard error (n = 3). Values with different letters are significantly different and the level of significant difference ($p<0.05$). ABTS scavenging activityexpressed as mg BHA equivalents per 100 g of fruit weight.

Superoxide scavenging activity of fruits

Table 4: Superoxide scavenging activity of fruits

Superoxide anion scavenging activity (mg AAE/100 g FW)	Methanol	Acidic- Methanol (pH 2)	Acetone	Acid- Acetone (pH2)
Rubus ellipticus	155.0±8.32	565.6±28.14	581.9±11.32	1083.0±2.23
E. oleraceae	267.87±6.17	872.52±28.92	357.16±17.32	876.04±20.02
V. myrtillu	17.98	28.1	31.57	26.33
P. embilica	782.99	371.13	1318.8	944.46
R. niveus	567.0	1139.85	1541.85	1611.6

Each value is expressed as mean ± standard error (n = 3). Each value is expressed as mean ± standard error (n = 3). Values with different letters are significantly different and the level of significant difference ($p<0.05$). Superoxide scavenging activity expressed as mg Ascorbic acid equivalents per 100 g of fruit weight.

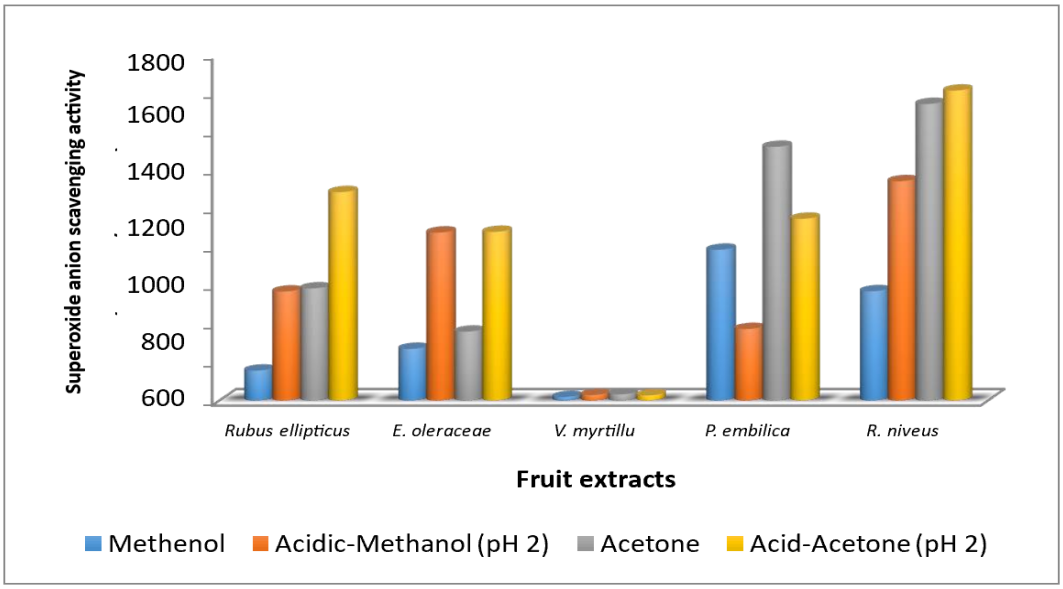

Figure 2: *Superoxide anion radical scavenging activity of wild edible fruits*

From the table 3 and table 4, it can be shown that most of the extract showed antioxidant activities.

Table 5: Antibacterial activity of standard antibiotics

Bacterial Strain	Zone of Inhibition (in millimeter)		
	Streptomycin(10 mcg/disc)	**Tetracycline (10 mcg/disc)**	**Chloramphenicol (10 mcg/disc)**
Escherichia coli (MTCC739)	18:o mm	20.0mm	21.0 mm
Bacillus subtilis (MTCC441)	12.0 mm	18.0 mm	19.0 mm
Staphylococcus aureus (MTCC96)	16.0 mm	18.0 mm	23.0 mm
Micrococcus luteus (MTCC106)	18.l	17.3	16.3

Table 6: Antibacterial activity of wild edible fruit extracts determined bymeasuring the zone of inhibition using paper disc (5 mm in diameter, Whatman No. 1) diffusion method (n = 4)

Bacterial test species	**Antibacterial activity of the fruitextracts (zone of inhibition in millimeter)**			
Rubus ellipticus	ReM	ReAM	ReA	ReAA
Escherichia coli (MTCC739)	ND	ND	ND	ND
Bacillus subtilis (MTCC944 7)	ND	ND	ND	ND
Staphylococcus aureus (MTCC96)	ND	ND	ND	ND
Micrococcus luteus (MTCC 106)	ND	ND	ND	ND
E. oleraceae	EoM	EoAM	EoA	EoAA
Escherichia coli (MTCC739)	ND	ND	ND	ND
Bacillus subtilis (MTCC9447)	ND	ND	ND	ND
Staphylococcus aureus (MTCC96)	ND	ND	ND	ND
Micrococcus luteus (MTCC 106)	ND	ND	ND	ND
V. myrtillu	VmM	VmAM	VmA	VmAA
Escherichia coli (MTCC739)	ND	ND	ND	ND
Bacillus subtilis (MTCC9447)	ND	ND	ND	ND
Staphylococcus aureus (MTCC96)	ND	ND	ND	ND
Micrococcus luteus (MTCC 106)	ND	ND	ND	ND
P. embilica	PeM	PeAM	PeA	PeAA
Escherichia coli (MTCC739)	ND	ND	ND	ND
Bacillus subtilis (MTCC9447)	ND	ND	ND	ND
Staphylococcus aureus (MTCC96)	ND	ND	ND	ND
Micrococcus luteus (MTCC 106)	ND	ND	ND	ND
R. niveus	RnM	RnAM	RnA	RnAA
Escherichia coli (MTCC739)	2±0.3	2.5±0.2	4.9±0.7	6.6 ±0.3
Bacillus subtilis (MTCC9447)	ND	ND	ND	ND
Staphylococcus aureus (MTCC96)	2±0.4	3±0.4	5±0.3	6.9±0.6
Micrococcus luteus (MTCC 106)	3.1±0.6	2.9±0.6	5.7±0.6	6.2±0.6

Conclusion

The obtained results revealed that all the tested species of wild Himalayan berries exhibited the ability to scavenge ABTS radical. Furthermore, the antioxidant potential of the methanolic and aqueous extracts of the berries was the most significant. It could be concluded from the study that the wild Himalayan berries could be a potential source of rich antioxidant molecules and may be helpful in obtaining new lead molecules for treating several conditions.

According to this study, all plant extracts contain interesting antibacterial characteristics that can be related to their abundance in phytochemicals, including phenolics and flavonoids.

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