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Research Article

Antioxidant and Antibacterial Activity of *Elaeagnus umbellata* from Rawalakot, Azad Kashmir

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ABSTRACT

Wild edible fruits (WEFs) that may be eaten are a good source of vitamins, proteins, and antioxidant enzymes are an important source of nutrients during times of hunger and can be used to prevent various illnesses. The objective of this study was to determine the metabolic and dietary profiles of *Elaeagnus umbellata* found in Tolipeer Azad Jammu and Kashmir. Total phenolic contents (TPCs) and total flavonoid contents (TFCs) of the fruit were tested. 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide (H_2O_2) scavenging assays were used to assess antioxidant activity. The antibacterial activity of *E. umbellata* against *Staphylococcus aureus* and *Pseudomonas aeruginosa* was examined using a disc diffusion approach. The E. umbellata methanolic extract had the highest levels of TPCs (23.00 g GAE/g) and TFCs (21.53 g QE/g). The methanolic extract of *E. umbellata* showed the strongest antioxidant activity against DPPH radicals (IC50 = 24.75 2.20 g/mL). *E. umbellata* displayed the strongest antibacterial activity against P. aeruginosa (15.00 ± 1.0 mm). It is concluded that *E. umbellata* could provide an alternative source of antioxidant, and antibacterial agents for controlling various types of infections and diseases.

Keywords: Antioxidant, Antibacterial, *Elaeagnus umbellata*.

INTRODUCTION

One of the most significant dietary sources of different antioxidant phytochemicals in the human diet is fruit. Obesity, diabetes, cardiovascular disease, cancer, and osteoporosis are among chronic illnesses that can be prevented with a good diet (Heber, 2009). Several fruit extracts and phytochemical groups have been discovered to exhibit significant antioxidant activity (Uddin et al., 2008). These fruits also produce medicine, fiber, fodder, and colors, among other things (Kayang, 2007). Fresh fruits are a key external source of antioxidants for humans, with polyphenols, flavonoids and vitamin C being the most common (Anwar *et al*., 2018).

Species that are neither domesticated nor cultivated but can be found in their natural habitat and are used as sources of food and socioeconomic well-being, particularly in rural underdeveloped areas, are known as wild edible fruits (WEFs). Millions of people in many developing countries lack access to adequate food to meet their daily needs, and many more are deficient in one or more micronutrients (Beluhan and Ranogajec, 2011; Sundriyal and Sundriyal, 2003). WEPs are receiving more attention since they include a variety of phyto components with strong antioxidant activity and health advantages (Harsha et al., 2013). The most significant ingredients are phenolics, flavonoids, and alkaloids, which are essential to their antioxidant capacity and the prevention of serious diseases including cancer and cardiovascular disease (Losso et al., 2007). Fruit eating has been linked to a lower risk of some chronic illnesses due to the

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presence of phytochemicals with various bioactivities.

Many fruits and vegetables contain L-ascorbic acid, the most physiologically active form of vitamin C. Vitamin C provides several health advantages, including antioxidant, cancer-fighting, and cardiovascular-protective properties (Ngo et al., 2019). Polyphenols, which comprise phenolic acids, flavonoids, proantho-cyanidins, stilbenes and lignin are the most prevalent phytochemicals in fruits. People love to eat wild edible fruits because of their natural origins and tasty flavor (Lorrain et al., 2013).

There are around 6000 distinct WEFs types, of which 600 are essential as nutritional ingredients (Abbasi et al., 2010). There are abundant populations of the deciduous shrub known as autumn olive (*Elaeagnus umbellata*) throughout the country's Himalayan regions (Sabir et al., 2007). The fruit of this plant is delicious, having phenols, flavonoids, vital fatty acids, vitamins, and minerals. It is believed that the fruit can stop cancer from starting and be utilized to stop it from spreading (Matthews, 1994). Considering the medicinal benefits and natural abundance of *E. umbellata*, the purpose of the current study was to assess the antioxidant and antibacterial activity of acetone and methanol extract of *E. umbellate*.

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MATERIALS AND METHODS

Plant material and preparation of extract

The fruits of *E. umbellate* were collected and cleaned with distilled water after being sun-dried. The fruits were first dried at 40°C in a hot air oven, then ground into a fine powder and stored in an airtight plastic container. To make powder, dried fruits were ground. The powdered samples were subjected to extraction with acetone and methanol (Sultana et al., 2009). About 10 g of powder was added in 50 mL of respective solvent and allowed for shaking (24h, 150 rpm, 30 °C) on an orbital shaker. All the extracts were then filtered and then filtrate was concentrated by rotary evaporator. The crude extract was used for phytochemical and biological investigation.

Total phenolic content

Using the Folin-Ciocalteu (FCR) technique, the total phenolic content was determined (Chang et al., 2002). Gallic acid was employed as the standard in this procedure. 100 mL of each plant sample was taken, 900 mL of distilled water was added and then H_2O was added back to the samples. Next, 0.5 mL of FCR was added to the mixture and 20 percent NA_2CO_3 solutions were created. 1.5mL of this solution was taken and added to the previous mixture (sample + water + FCR). The entire combination was simmered for about two hours in a volumetric flask. A UV-vis spectrophotometer was used to measure the mixture's absorbance at 720 nm after it had cooled. Total phenolic contents in samples were assessed by using gallic acid equivalent (GAE) as standard.

Total flavonoid content

By using the aluminum chloride colorimetric method, total flavonoid was calculated (Sakanaka et al., 2005). 75 mL of a 5 percent (w/v) sodium nitrite solution and 1.25 mL of distilled water were added to 200 L of (mg/mL) extract and incubated for 6 minutes. The reaction mixture was then mixed with 150 L of a 10 percent (w/v) aluminum trichloride solution, and it was allowed to sit at room temperature for 5 minutes. Then, immediately following the addition of 0.5 mL 8 of 1M sodium hydroxide solution, the absorbance was measured at 510 nm. Quercetin equivalent per gramme (QE/g) of the plant material was used to express the amount of total flavonoid.

Antioxidant activity

The method used to test the chard extract's capacity to scavenge DPPH free radicals was approved and described by (Dias et al., 2014) with a few minor adjustments. About 1 mL of the freshly obtained ethanol extract was combined with 2 mL of a DPPH solution in various strengths (0.2 mM in methanol). The mixture was incubated at 25°C for 30 minutes, and a UV-vis spectrophotometer (Perkin Elmer Lambda 40 UV/VIS Spectrophotometer) was used to measure the absorbance at 517 nm. Using the following equation, the effectiveness of radical scavenging was calculated as a percentage of DPPH radical elimination:

$$\%\ scavenging = \frac{control - sample}{control} \times 100$$

Antibacterial activity

Each sample's methanol and acetone extract was evaluated independently using the Disc-diffusion assay for bacterial resistance**.** For a final concentration of 30 mg/mL, the extracts were dissolved in the same solvent (methanol and water). Then, disc diffusion antibacterial tests were performed using 0.1 mL of a solution containing 108 CFU/mL of bacteria dispersed on nutritional agar**.** The discs (6 mm in diameter) were placed on the inoculated agar after being impregnated with 30 mg/mL extracts (300 g/disc). The same solvents used to dissolve the plant extracts were also used to generate negative controls. For each investigated microbial species, one strain or isolate

was evaluated for sensitivity to ampicillin using positive reference standards. The plates with the inoculum were incubated for 24 hours at 37°C. The zone of inhibition against the test pathogens was measured to assess the antibacterial activity. In the experiment, each assay was conducted twice.

Statistical analysis

Triplicates of the experiments will be run. Analysis of variance (ANOVA) will be used to analyse the results, and the means will be compared using the least significant difference method (LSD).

RESULTS

Total phenolic and total flavonoid contents

The presence of TPCs and TFCs in the methanol and acetone extract of *E. umbellate* was qualitatively assessed (Table 4.1). The methanol extract of *E. umbellata* showed higher phenolic (23.00 g GAE/g) as compared to acetone extract (21.3 g GAE/g). Similarly, the flavonoid contents were found higher in methanol extract (21.53 g QE/g) than acetone extract (20.44 µg QE/g (Table 1). According to Kahkonen *et al*. (1999), the phenolic compounds, which have a very high redox potential, perform the functions of hydrogen donors, reducing agents, and singlet oxygen quenchers. The majority of naturally occurring antioxidants come from plants in the form of phenolic chemicals such flavonoids and phenolic acids (Ali *et al.,*2008). There is a clear correlation between total phenol content and antioxidant activity, and plants with higher phenolic content exhibit good antioxidant activity (Brighente *et al*., 2007 and Salazar *et al*., 2008). Phenols contain qualities including anti-aging, anti-inflammation, and anti-apoptosis, claimed by Yaday and Agarwaly (2014). Many bacterial strains are known to be inhibited by or killed by flavonoids and some pathogenic protozoans are also known to be destroyed by flavonoids. (Chantal *et al*., 2005). Additionally, flavonoids are easily eaten by people and have been shown to have significant anti-cancer, anti-inflammatory and anti-allergenic effects (Crozier *et al*., 2006). They are widely distributed in plants in the form of their glycosides (Rajanandh and Kavitha, 2010).

Table 1: Total Phenolic and flavonoid contents in *Ficus palmata, Elaeagnus umbellata,* and *V, vinifera* with acetone and methanol extracts.

Plants	Extracts	Total phenolic content (µg GAE/g)	Total Flavonoid contents (µg QE/g)
E. umbellata	Acetone	21.3	20.44
	Methanol	23.00	21.53

Antioxidant activity

Utilizing DPPH scavenging activity, three wild edible fruits' free radical scavenging activity was discovered. In comparison to ascorbic acid, Table 4.2 illustrates the antioxidant activity of different concentrations of *E. umbellate* on DPPH free radicals in terms of percentage inhibition. The findings showed that extracts of this plant have concentration-dependent scavenging activity. When compared to normal ascorbic acid, both extracts at varied concentrations (1000, 500, 250, 125 µg/ml) showed significant inhibition of DPPH radicals. *E. umbellata* with methanol extract revealed higher antioxidant activity with 30.29**±**1.05 % antioxidant activity against DPPH (Table 2). Antioxidant activity was directly correlated with the amount of total phenolic contents in the fruit extracts (Mensour *et al*., 2011).

Table 2: Antioxidant activity of *Ficus palmata, Elaeagnus umbellata,* and *Vitis vinifera* against DPPH in different solvents.

Plants	Solvents	Antioxidant activity (%) ± standard error of means			
		1000 µg/mL	500 µg/mL	250 µg/mL	125 µg/mL
E. umbellata	*Acetone*	25.11±5.32	31.21±1.11	29.3±1.9	26.37±1.23
	Methanol	30.29±1.05	24.01±2.33	23.4±0.05	22.81±1.66
Ascorbic acid		88.05±5.23	87.8±4.93	86.32±3.91	75.86±1.75

A potent oxidizing agent, hydrogen peroxide (H_2O_2) can activate the signaling pathway to promote cellular proliferation or differentiation. In a biological system, it is produced by a variety of oxidizing enzymes, including superoxide dismutase. However, oxidative stress and inflammatory responses are caused by abnormal H_2O_2 buildup, and these reactions are linked to pathological disorders such cancer, diabetes, and cardiovascular illnesses. This is because H_2O_2 decomposes quickly, producing the hydroxyl radical (•OH), which then starts the oxidation of lipids and damages cellular components. In biological research, controlling H_2O_2 production by plant antioxidants is of great interest.

Antibacterial activity

Antibacterial activity of selected wild edible fruits was tested against *S. aureus* and *P. aeruginosa* bacteria through disc diffusion method. While acetone extract was shown to be equally effective in stopping the growth of *P. aeruginosa* and *S. aureus*, methanol extract only displayed modest antibacterial action against bacteria*. E. umbellate* showed 13.44±1.06 mm zone of inhibition against *S. aureus* and 14.23±1.03 mm against *P. aeruginosa* (Table 3). The MeOH extract showed good antimicrobial activity at 100 mg/ml and was more potent towards wild fruits (Jagtap Supriya et al., 2012). One of the typical medicinal plants utilized by local practitioners to treat a variety of human problems is *Elaeagnus umbellata*, but no attempt has been made to investigate its antimicrobial potential. When compared to other solvents, the methanol extract, however, demonstrated the greatest inhibition against all tested species. This shows that the fruit of *E. umbellata* is highly effective at killing gram-positive and gram-negative bacteria. Only *Pseudomonas aeruginosa* was resistant to the plant's aqueous extract, which inhibited 99 percent of the microbes. Since gram-negative bacteria are often less resistant than this class of bacteria, *Pseudomonas aeruginosa's* resistance to plant extract was not surprising. Such resistance can result from the cell wall's permeability barrier or the membrane accumulation mechanism*. Pseudomonas aeruginosa* infections, particularly those with multi-drug resistance, are some of the most challenging to treat with conventional antibiotics. Acetone extract showed significant anti-*Pseudomonas aeruginosa* action in this investigation. Therefore, it appears very likely that the antibacterial compound obtained from *E. umbellata* may inhibit bacteria by a different mechanism than that of currently prescribed antibiotics and may be useful as a therapeutic antibacterial agent against bacterial strains that have developed a resistance to multiple drugs (Adwan and Abu-Hasan, 1998). It was also shown that the phospholipid and glycolipid fatty acid composition of *E. umbellata* fruit may also be the cause of the antibacterial activity of berry extracts (Goncharova et al., 1993).

Table 3: Antibacterial activity of *E. umbellata* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Bacteria	Zone of inhibition (mm) ± Standard error of means	
	Ampicillin	*E. umbellata*
S. aureus	14.66±1.09	13.44±1.06
P. aeruginosa	13.66±1.01	14.23±1.03

Conclusion

It is concluded that the knowledge about the use of wild fruits is only in the remembrance of elderly people, and it is vanishing. Further research should be done on these plants to find ways to combat protein and energy deficiency, especially in underdeveloped nations. Utilizing these plants for the development of novel foods and for use in the pharmaceutical business will undoubtedly be beneficial if contemporary processing techniques are used in conjunction with traditional expertise. However, there is still much need for investigation into wild species and evaluation of the nutritious qualities of these wild resources. Wild fruits are useful in the development of novel medicines and in the prevention of illnesses.

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AUTHOR CONTRIBUTIONS

All authors contributed equally to this research.

COMPETING OF INTEREST

The authors declare no competing interests.

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