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Original Article

ANTIOXIDANT, ANTIMICROBIAL ACTIVITY AND TOTAL PHENOL AND FLAVONOIDS ANALYSIS OF *SAMBUCUS NIGRA* (ELDERBERRY)

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ABSTRACT

Objective: The goal of this study was paying attention on the chemical characterization of phytochemical compounds and their antioxidant activity of *Sambucus nigra* L.

Methods: Phytochemical analysis was performed by *Sambucus nigra* L fruit extract. Total Phenol and Flavonoids content of elderberry fruit extract also determined using Folin-Ciocalteu colorimetric method and aluminum chloride colorimetric method. Antibacterial activity was performed by disk diffusion method and Antioxidant capacity was investigated by DPPH assay, butylhydroxytoluene used as a standard.

Results: The richest anthocyanin in elderberry fruits was cyanidin-3-*O*-sambubioside. The antioxidant capacity obtained for elderberry extract proved that elderberry shown highest antioxidant activity, being the richest anthocyanins. The antioxidant capacity of elderberry fruit methanolic extract was recorded 62.56±1.12 percentages of scavenging activity. We also investigated antibacterial activity against four species *Escherichia coli, Pseudomonas putida, Bacillus cereus,* and *Staphylococcus aureus.* There E. coli was recorded 12.0 mm and *Pseudomonas putida* was recorded 0.34 mm zone of inhibition.

Conclusion: The conclusion of our study is that *Sambucus nisgra* fruit extract has very high antioxidant activity which makes it recommendable for food industry and dietary supplement.

Keywords: Antioxidant, Phytochemical, *Sambucus nigra,* Anthocyanin, Antimicrobial activity

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INTRODUCTION

Sambucus nigra L. belong to *Adoxaceae* family, which growing on sunlight-exposed locations in Europe. *Sambucus nigra* L. currently, more consideration has been focused on natural compounds that may valuable in dropping oxidative stress-induced diseases. Elderberries contain flavonols glycosides, organic acids and anthocyanins [1, 2]. However, the anthocyanins present in elderberries are showing important for favorable health effects related with their antioxidant activity and their capability has been recorde to protect against influenza A and B virus, colon cancer respective *Helicobacter pylori* infections [3-5]. There are numerous studies behind the idea that elderberry is an accurate pharmacy, measured to be an abundant source of bioactive components, which it is offered in modern medicine.

The elderberry contains numerous compounds that may supply to pharmacological activities. High amounts of anthocyanins like cyanidin-3-sambubioside and cyanidin-3-glucoside. Less amounts of other kinds of flavonols, anthocyanins, phenolic acids and procyanidins have also been investigated [6]. Some other components were also identified such as vitamins, minerals carbohydrates include 7.5% glucose, pectin and fructose [7].

The consumption of elderberry helps to prevent and therapy for many diseases, like diabetes, obesity, immune system stimulation, antibacterial and antifungal activity, antitumor activity, diuretic and laxative activity, protection against UV radiation [8-14].

Bioactive compounds of plants responsible for their antimicrobial properties include alkaloids that damage the DNA of microbial cells and most important to cell death [15]. The major components are liable for the antimicrobial properties of plants, which is polyphenolic compound. They display anti-inflammatory activity *in vitro* and *in vivo* and mechanism of the inhibition of enzymes [16, 17].

The antioxidant activity of elderberry anthocyanins and flavonols is due to the being there of *meta* 5,7-dihydroxy arrangements in the A ring and *ortho* 3',4'-dihydroxy moiety in the B ring [18]. Phenolics compounds present in berries is affected only by genetic differences, environmental conditions, degree of maturity is most important for pharmaceutical industry to know the chemical composition and the antioxidant capacity [19].

The purpose of this study was to establish the phytochemical screening, total phenol and Flavonoids content analysis and antimicrobial effects of elderberry fruit. Antioxidant properties of elderberry fruit also investigated.

MATERIALS AND METHODS

Materials and chemicals

The elderberry fruits was purchased from traditional local suppliers of medicinal herbs. gallic acid, anhydrous sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH., 95%) were obtained from sigma-aldrich (Steinheim, Germany). Folin-ciocalteu phenol reagent, methanol, acetone were procured from merck.

Extract preparation

50 g of dried fruit powder was soaked in 200 ml of methanol for 48 h. The extract was filtered and evaporated at the room temperature. Obtained residues were resuspended in methanol.

Bacterial strains

The bacterial isolates *Escherichia coli, Pseudomonas fluorescens, Bacillus cereus,* and *Staphylococcus aureus* were procured from MTCC Chandigarh. The bacteria were maintained on nutrient agar.

Phytochemical screening

The phytochemical analysis of phenolics, terpenoids, phytosterols, flavonoids, carbohydrates, glycosides, saponins tannins and alkaloids in methanol elderberry extracts were performed using previously described procedures of A. Sofowora [20].

Total phenolic content

The total phenol content was determined by the Folin-Ciocalteu colorimetric method with some modification [21]. Briefly, 0.025 ml of plant extract, 0.125 ml of Folin-Ciocalteu reagent and 1.975 ml of distilled water were mixed carefully. After 3 min, 0.375 ml 20 % sodium carbonate was added. Mixture was incubated in dark condition, at room temperature for 2 h. the observance was recorded at 750 nm. The results were expressed as mg of gallic acid equivalents (GAE)/g DW. gallic acid used as a standard. All tests were done in triplicates.

Total flavonoid content

The total flavonoid content was determined using the aluminum chloride colorimetric method [22]. Briefly, 0.25 ml of extract was added to 1 ml distilled water. Then 5% of 0.075 ml of sodium nitrite was added. After 5 min, 10% of 0.075 ml of aluminum chloride was added. After 6 min, 0.5 ml of 1 mol/l sodium hydroxide solution and the volume was makeup to 2.5 ml with distilled water. The absorbance was recorded at 510 nm. Rutin used as a standard. The results were expressed as mg of rutin equivalents (RE)/g DW.

Antimicrobial activity

The antibacterial activity of the methanolic extract was investigated by the disk diffusion method, on Mueller Hinton Agar medium. The experiment was performed by using 24 h old bacterial suspension. The extract was tested using 5 mm sterilized filter paper discs. The discs were impregnated with 20 µl of the extract, kept to dry under laminar airflow and then placed into previously inoculated Petri dishes. Subsequently, the plates were incubated for 24 h at 37 °C. Standard antibiotic discs used as positive controls for the antimicrobial activity. After incubation, the diameter zone of inhibition was measured.

DPPH radical scavenging activity

The DPPH assay was performed previously describe method by Brand-Williams [23]. Briefly, 1 ml of DPPH solution was allowed to react with 1 ml extract. The capacity of the polyphenolic compounds, which is act as free radical scavengers against DPPH radical. After 30 min at 40 °C the absorbance was recorded at 517 nm, against a blank. Standard curve was organized using diverse concentrations of butylhydroxytoluene and the results were presented as the percent of control.

The antioxidant activity was calculated as follows:

% DPPH scavenging activity = (1-[Asample/Acontrol $_{t=0}$]) 100

RESULTS

Phytochemical screening

The phytochemical screening result of elderberry methanol extract revealed the presence of phenols, flavonoids, tannins, anthocyanin and carbohydrates. There alkaloids and saponins were not detected (table 1).

Total phenols and flavonoids content

The most important groups of compounds being responsible for antimicrobial activity include Phenolics and Flavonoids. The variation in the antimicrobial effect that may become from the variations in the structure and chemical composition of these compounds.

The total phenolic content elderberry was determined using folin-ciocalteau method. TPC was recorded 43±0.98 mg GAE/g DW. The total Flavonoid content was recorded 15±1.12 mg rutin equivalents/g DW (table 2).

Antibacterial activity

Antimicrobial activity of elderberry fruit extract were presented in table 3. Antibiotic effect depended on the solvent used for extraction. After eliminating, most important zone of inhibition found against (12.0 mm) *E. coli*. Elderberry methanol extract also shown less zone of inhibition (0.33 mm) against *Pseudomonas pudita*. However, *Bacillus cereus* and *Staphylococcus aureus* were not recorded inhibition zone. Antibacterial activity results were presented in table 3.

Antioxidant activity DPPH assay

The antioxidant activity of elderberry fruit extract was determined by DPPH assay. Antioxidant activity result was presented in table 4. The method used is for the reduction of the radical DPPH in the presence of hydrogen donating antioxidants.

Table 1: Phytochemmical screening of elderberry fruit

Phytochemical compounds	Methanol extract
Alkaloids	-ve
Phenols	+ve
Flavonoids	+ve
Tannins	+ve
Saponins	-ve
Anthocyanin	+ve
Sterols	+ve
Carbohydrates	+ve

Table 2: Total phenol and flavonoids content analysis of elderberry fruit extract

Extract	Total phenols content	Total flavonoids content
Methanol extract	43±0.98 mg GAE/g DW	15±1.12 mg rutin/g DW

Table 3: Antibacterial activity of methanol extract of elderberry fruit

Bacterial name	Zone of inhibition (mm)
Escherichia coli	12.0
Pseudomonas pudita	0.34
Bacillus cereus	-
Staphylococcus aureus	-

Table 4: Antioxidant activity of elderberry fruit methanol extract

Extract	DPPH radical scavenging activity (%)
Methanol extract	62.56±1.12

CONCLUSION

Elderberries are predominately used as food and dietary supplements, for that reason it is very important useful to know their compounds such as phenolic and flavonoids. *Sambucus nigra* contained mainly anthocyanins as cyanidin 3-*O*-sambubioside and cyanidin 3-glucoside. In this study we conclude phytochemical analysis, Total Phenol and Flavonoids and antibacterial activity against four species.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

Declared none

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