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. Antimicrobial 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 62.5±1.12 percentages of scavenging activity. We also investigated antimicrobial 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 nigra 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
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, 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]) 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 (0.3 mm) against Pseudomonas pudita. However, Bacillus cereus and Staphylococcus aureus were not recorded inhibition zone. Antimicrobial 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.

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### Table 1: Phytochemical screening of elderberry fruit

<table>
<thead>
<tr>
<th>Phytochemical compounds</th>
<th>Methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-ve</td>
</tr>
<tr>
<td>Phenols</td>
<td>+ve</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+ve</td>
</tr>
<tr>
<td>Tannins</td>
<td>+ve</td>
</tr>
<tr>
<td>Saponins</td>
<td>-ve</td>
</tr>
<tr>
<td>Anthocyanin</td>
<td>+ve</td>
</tr>
<tr>
<td>Sterols</td>
<td>+ve</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+ve</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total phenols content</th>
<th>Total flavonoids content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol extract</td>
<td>43±0.98 mg GAE/g DW</td>
<td>15±1.12 mg rutin/g DW</td>
</tr>
</tbody>
</table>

### Table 3: Antimicrobial activity of methanol extract of elderberry fruit

<table>
<thead>
<tr>
<th>Bacterial name</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>12.0</td>
</tr>
<tr>
<td>Pseudomonas pudita</td>
<td>0.34</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>-</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table 4: Antioxidant activity of elderberry fruit methanol extract

<table>
<thead>
<tr>
<th>Extract</th>
<th>DPPH radical scavenging activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol extract</td>
<td>62.5±1.12</td>
</tr>
</tbody>
</table>
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

REFERENCES