PHENOLIC CONSTITUENTS, FLAVONOID CONSTITUENTS, ANTIOXIDANT, AND TOXICITY OF ETHANOL EXTRACT OF ROOT, STEM, LEAF, FLOWER, FRUIT, AND SEED OF Gynandropsis gynandra (L.) Briq.

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ABSTRACT

Gynandropsis gynandra (L.) Briq. in Palu, Central Sulawesi, is used as a food ingredient and empirically used in traditional medicine. This study determined the phytochemical content, phenolic, and flavonoid constituents, antioxidants, and toxicity of a 96% ethanol extract of G. gynandra of the root, stem, leaf, flower, fruit, and seed. Maceration was used to get G. gynandra extract. Standard methods for phytochemical screening of extracts were used. Determination of phenolic constituents using Folin-Ciocalteau, and flavonoid constituents using AlCl₃, then determined by UV-Vis Spectrophotometry. The DPPH method was used to determine antioxidant activity and the IC₅₀ value. The toxicity test used the Brine Shrimp Lethality Test (BSLT) and determined the LC₅₀ value. Phytochemical screening showed that all 96% ethanol extracts contained alkaloids, flavonoids, saponins, and tannins. The highest antioxidant activity (IC₅₀) was 96% ethanol extract of leaf 116.6 µg/mL with total phenolic 16.821 ± 0.836 mg GAE/g extract and total flavonoid 21.956 ± 2.986 mg QE/g extract. The highest lethal concentration of 50% (LC₅₀) was 96% ethanol extract of seed 26.30 µg/mL. According to the study results, the 96% ethanol extract of the leaf and seed of G. gynandra, respectively, has the potential to be evaluated as an antioxidant and anticancer agent.

Keywords: Gynandropsis gynandra (L.) Briq., Total Phenolic, Total Flavonoid, Antioxidant, Toxicity.

INTRODUCTION

Free radicals are reactive molecules produced by various processes in the body, such as metabolism, cellular respiration, and inflammatory reactions. Free radicals do not only come from within the body, but can also be generated from environmental pollution, excessive exposure to UV light, γ-ray radiation, X-ray radiation, and cigarette smoke. At high concentrations, free radicals can oxidize cell components such as nucleic acids, proteins, fats, and DNA to initiate diseases such as hypertension, atherosclerosis, nervous disorders, diabetes, asthma, aging, and even cancer.¹,² Antioxidants are compounds that can counteract and prevent damage caused by free radical compounds.³ Sources of antioxidants can be synthetic antioxidants and natural antioxidants. Animal studies demonstrate that synthetic antioxidants like Butylated hydroxyanisole and Butylated hydroxytoluene might cause liver and renal malfunction, allergic responses, and carcinogenesis. Therefore, more and more research is being carried out on natural ingredients with antioxidant activity that are not toxic.⁴,⁵ Flavonoids are the most common phenolic compounds, as they are widely distributed in plant tissues and are responsible for giving plants their colour. Flavonoids have a 15-carbon skeleton consisting of two substituted benzene rings linked by a three-carbon aliphatic chain.⁶ Phenol molecules can operate as antioxidants by reacting with reactive oxygen species (ROS) and neutralizing their radical activity, making them no longer damaging to human cells.⁷ These phenolic chemicals can help prevent heart disease, inflammation, cancer, and diabetes and lower the level of mutagenesis in human cells. The protection provided by plant products, including fruits, vegetables, and...
nuts, is largely due to these plants' phenolic chemicals. Previous studies have shown that the ethanol extract of *Gynandropsis gynandra* (L.) Briq. herb has antioxidant activity (IC$_{50}$ 189.455 µg/mL) and toxicity (LC$_{50}$ 472.648 mg/L). This study aimed to determine the antioxidant activity and toxicity of a 96% ethanol extract of *G. gynandra* of the root, stem, leaf, flower, fruit, and seed to obtain scientific information for developing plants in the health sector, especially pharmaceuticals.

**EXPERIMENTAL**

**Plant Materials and Extraction Procedure**

*G. gynandra* was obtained from cultivated plants in Sigi, Central Sulawesi, Indonesia. This plant was identified by the Biodiversity Laboratory, Department of Biology, FMIPA Tadulako University, with voucher number 109/UN.28.UPT-SDHS/LK/2020. *G. gynandra* simplicia powder was macerated in 96% ethanol at a ratio (1:10 w/v) for 3x24 hours. A rotary vacuum evaporator was used to concentrate the filtrate, which was then concentrated further in a water bath at 50°C to produce a dry extract.

**Phytochemical Screening of Secondary Metabolites**

Phytochemical screening of secondary metabolites of alkaloids, phenolics, flavonoids, and saponins using standard methods for qualitative tests.

**Determination of Total Phenolic Content**

The total phenolic content was determined using the Folin-Ciocalteau reagent with slight modifications. 10 mg of the extract was dissolved in 10 mL of 96% ethanol to obtain a 1000 µg/mL concentration, and 0.5 mL of the test sample was added to 5 mL of Folin-Ciocalteau reagent (1:10) and 4 mL of 1 M sodium carbonate. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 735.3 nm after 15 minutes of incubation. A gallic acid calibration curve with a linearity range of 5-160 µg/mL and $R^2$=0.9951 was used to calculate total phenolic content in milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g DE).

**Determination of Total Flavonoid Content**

The total flavonoid content was determined using the Aluminum chloride test with slight modifications. 10 mg of the extract was dissolved in 10 mL of 96% ethanol to obtain a concentration of 1000 µg/mL, 1.5 mL 96% ethanol, 0.1 mL 10% aluminum chloride, 0.1 mL potassium acetate 1 M, and 2.8 mL distilled water were added to 0.5 mL of the test sample. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 444.8 nm after 30 minutes of incubation. A quercetin calibration curve with a 5-80 µg/mL and linearity range $R^2$=0.9905 was used to calculate the total flavonoid content in milligrams of quercetin equivalent per gram of dry extract (mg QE/g DE).

**Antioxidant Activity**

The antioxidant activity of the extract was determined using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical reduction activity. Five different concentrations of the extract were dissolved in methanol. 1 mL of extract was mixed with 1 mL of 0.1 mM DPPH, incubated for 30 minutes at room temperature, and in a dark area. A UV-Vis spectrophotometer was used to measure the absorbance of the solution at a wavelength of 515.3 nm. The blank solution was pure methanol, and the control solution was DPPH. The formula was used to compute the percentage of DPPH radical inhibition from each concentration of sample solution:

$$\text{Inhibition} (%) = \left[ \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \right] \times 100$$

The 50% inhibitory concentration (IC$_{50}$) was calculated by plotting the percentage inhibition against the sample concentration. Quercetin and Vitamin C were used as standards.

**Toxicity Test**

*Artemia salina* eggs are inserted into a container filled with filtered salt water. Continue to expose the eggs to light for another 48 hours until they hatch perfectly. The ethanol extract was dissolved in salt water to obtain a 10-500 µg/mL concentration. The extract solution was then introduced to a vial with 10 shrimp larvae, which were allowed at room temperature for 24 hours before counting the number of living larvae.
Three replications were used in the experiment. The correlation curve between the log10 extract concentration (x-axis) and the probit value (y-axis) was used to establish the lethal concentration of 50% (LC$_{50}$) (Fig.-2).

RESULTS AND DISCUSSION

Phytochemical screening test showed that 96% ethanol extract of root, stem, leaf, flower, fruit, and seed of *G. gynandra* contained alkaloids, flavonoids, saponins, and tannins (Table-1). These compounds are known to exhibit medical and physiological activities. Alkaloids are reported to have antispasmodic and antibacterial activity.\textsuperscript{17} Flavonoids have antiviral, antioxidant, anti-atherosclerosis, anti-inflammatory activities, anticancer, and prevent neurodegenerative diseases.\textsuperscript{18-21} Saponins are known to have anti-inflammatory activity.\textsuperscript{22} Tannins are antifungal, antibacterial, and antiviral, according to studies.\textsuperscript{23} The maceration extraction of each portion of the *G. gynandra* plant revealed that the 96% ethanol extract of the leaf and fruit of *G. gynandra* produced the maximum yield (Table-1). These results indicate that the maceration method and 96% ethanol solvent were more optimal in extracting compounds for the leaf and fruit of *G. gynandra*.

Table-1: Phytochemical Screening of 96% Ethanol Extract of *G. gynandra*

<table>
<thead>
<tr>
<th>Phyto-constituents</th>
<th>Test performed</th>
<th>Parts</th>
<th>Root</th>
<th>Stem</th>
<th>Leaf</th>
<th>Flower</th>
<th>Fruit</th>
<th>Seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>Dragendorff's</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>Shinoda</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>Saponin</td>
<td>Froth formation</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>Tannin</td>
<td>FeCl$_3$</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>Yield (%)</td>
<td>3.4</td>
<td>4.3</td>
<td>6.21</td>
<td>3.60</td>
<td>6.06</td>
<td>3.17</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The 96% ethanol extract of *G. gynandra* has the highest total flavonoid content in the stem and leaf (Table-2). flavonoids are antioxidant chemicals found in nature that are soluble in polar solvents, including water, methanol, and ethanol. Water, methanol, and ethanol are easily soluble in flavonoid glycosides, whereas aglycone flavonoids are exclusively soluble in methanol and ethanol. The solvent utilized in this study was 96% ethanol. The extracted flavonoid compounds were flavonoid glycosides and aglycones based on the type of solvent used.\textsuperscript{24-25} The total phenolic content of the 96% ethanol extract of *G. gynandra* was determined, and the results showed varied total phenolic levels (Table-2). Several studies have shown that solvents significantly affect variations in phenolic and flavonoid content.\textsuperscript{26,27}

Table-2: Total Phenolic and Total Flavonoids of 96% Ethanol Extract of *G. gynandra*

<table>
<thead>
<tr>
<th>Extract of <em>G. gynandra</em></th>
<th>Total Phenolic (mg GAE/g extract)</th>
<th>Total Flavonoid (mg QE/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem</td>
<td>12.509 ± 0.262</td>
<td>23.937 ± 0.726</td>
</tr>
<tr>
<td>Leaf</td>
<td>16.821 ± 0.836</td>
<td>21.956 ± 2.986</td>
</tr>
<tr>
<td>Flower</td>
<td>14.676 ± 2.792</td>
<td>9.590 ± 1.072</td>
</tr>
<tr>
<td>Fruit</td>
<td>11.287 ± 0.484</td>
<td>8.333 ± 0.053</td>
</tr>
<tr>
<td>Seed</td>
<td>14.408 ± 0.685</td>
<td>10.998 ± 0.198</td>
</tr>
</tbody>
</table>

GAE: Gallic Acid Equivalent; QE: Quercetin Equivalent; IC$_{50}$: inhibitory concentration 50%
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The leaf of *G. gynandra* had the highest antioxidant activity (IC$_{50}$), but the antioxidant activity was about 7 times lower than that of vitamin C and quercetin (Fig.-1). The antioxidant activity of each part of *G. gynandra* showed varied activity. These results were related to differences in the extract's total phenolic and total flavonoid content. The high antioxidant activity of *G. gynandra* leaf was associated with higher flavonoid and phenolic content than other parts.

<table>
<thead>
<tr>
<th>Extract of <em>G. gynandra</em></th>
<th>LC$_{50}$ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td>190.55</td>
</tr>
<tr>
<td>Stem</td>
<td>169.82</td>
</tr>
<tr>
<td>Leaf</td>
<td>162.18</td>
</tr>
<tr>
<td>Flower</td>
<td>199.53</td>
</tr>
<tr>
<td>Fruit</td>
<td>51.29</td>
</tr>
<tr>
<td>Seed</td>
<td>26.30</td>
</tr>
</tbody>
</table>

The lethal concentration of 50% (LC$_{50}$) of the 96% ethanol extract of *G. gynandra* was < 200 mg/L (Table-3). The LC$_{50}$ value of 96% ethanol extract of *G. gynandra* was < 1000 mg/L, indicating that it was harmful to *A. salina* larvae. These findings suggest that the 96% ethanol extract of *G. gynandra*, particularly the seed (LC$_{50}$ < 30 µg/mL), might be examined further for anticancer activities. This activity is thought to be due to the content of alkaloid compounds and flavonoids in the extract.

**CONCLUSION**

The 96% ethanol extract of *G. gynandra* of the root, stem, leaf, flower, fruit, and seed contains various flavonoids. The 96% ethanol extract of *G. gynandra* leaf had the highest antioxidant activity. The 96% ethanol extract of *G. gynandra* of each plant part, especially the seed part, has the potential as an anticancer agent. Isolation of the actual chemical compound responsible for its activity and mechanism requires further investigation to develop this plant, especially in herbal medicine.

**ACKNOWLEDGEMENT**

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