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Physical and Phytochemical Analysis of *Justicia gendarussa Burm. F.* Leaf Extracts

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Abstract

Objectives:

Methods: An experimental research design was used in this study based on phytochemicals screening, which includes the detection of major secondary metabolites. A qualitative analysis was done on the phytochemicals aspect, while qualitative and quantitative analyses were used to determine the physical properties of the plant extract. *J. gendarussa* leaf extract was subjected to physical examination to determine its (a) boiling point, (b) colour, (c) density, (d) odour, (e) pH and (f) miscibility. Phytochemical analysis of the extract was carried out to determine the presence of the following compounds: (a) alkaloid, (b) anthraquinone, (c) leucoanthocyanin, (d) phenolic compounds, (e) saponin, (f) steroid, (g) tannins and (h) terpenoids. Percent yield was also computed on the leaf extracts of *J. gendarussa*. **Results:** From the observed physical properties, it was generalised that the *J. gendarussa* leaf extract has a 103.3°C boiling point, a brown colour and pleasant odour, 1.02 g/mL of density, a neutral pH of 7 and is a polar substance. By weighing the collected extracts of *J. gendarussa* and dividing it by its weight of its leaves, the percent yield was recorded to be at 30%. It was documented by collecting about 30 g of crude *J. gendarussa* extract from the total 100 g of leaves used in the conduct of this study. Phytochemical analysis showed that only alkaloid and saponin are present in the plant extract of *J. gendarussa* leaves, and other secondary metabolites such as terpenoids, tannin, anthraquinone, leucoanthocyanin, phenolics and steroids were found to be absent in the plant extract. **Improvements/further study:** It is suggested that a further study should be conducted to characterise and quantify alkaloid and saponin in the extract of *J. gendarussa*. Also, a study using various methods of extraction and solvents is important to determine if there is a difference in secondary metabolite screening as well as to make use of *J. gendarussa* as a natural source of alkaloid and saponin.

Keywords: *J. gendarussa*, Chemical Analysis, Phytochemistry, Plant-derived Substances

1. Introduction

Most of the plants growing in our surroundings are ignored especially those that are wild. In the Philippines, some of these unnoticed plants, including *Justicia gendarussa Burm. F.*, can be used to cure or prevent certain diseases.

Justicia gendarussa Burm. F. is a native plant to China, Sri Lanka, Malaysia, India and the Philippines. It is found at low to medium altitudes and is rarely cultivated. *Justicia gendarussa Burm. F.* is shade-loving, quick-growing, erect, branched evergreen shrub measuring 0.6–1.2 m in height. The leaves are simple, opposite, lanceolate or linear-lanceolate, acute at base, tapering into rounded apex, and glabrous shining (8–12.5 cm long, 1.2–2 cm broad) with prominent purple veins beneath. The stem is quadrangular, thickened at and above the nodes and internodes measuring 2–7 cm long. The flowers are in terminal or axillary spikes and are irregular, bisexual, sessile, white with pink or purple spots inside, red in the throat and lip.[1] The leaf of *J. gendarussa Burm. F.*

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is sometimes applied to treat muscle pains, broken/fractured bone and boils.[2]

J. gendarussa is used in a certain province in the Philippines to ease the pain of women after childbirth. Folks believe that it prevents the "*bughat*" or the susceptibility of the mother to illnesses and diseases. Also, *J. gendarussa* is used as a remedy to pains in muscles and fractured bone.[3] Hence, it is important to study the phytochemicals present in the leaf extracts of *J. gendarussa Burm. F.* to endorse it as a plant that can be used to treat health problems.

2. Objectives of the Study

This study was conducted to detect the phytochemicals present in the leaf extract of *J. gendarussa Burm. F.*; to determine the physical properties of *J. gendarussa Burm. F.* leaf extract in terms of boiling point, colour, density, odour, pH, and miscibility; as well as to determine the percent yield of the leaf. Secondary metabolites present in *J. gendarussa Burm. F.* leaf extracts, including alkaloid, anthraquinone, leucoanthocyanin, phenolic compounds, saponin, steroid, tannins, and terpenoids, were also investigated.

3. Methodology

3.1. Research Design

An experimental research design was used in this study. Physical testing and percent yield of the plant extract was also done and computed. Simple mean and average statistical tools were used to assess certain physical properties.

3.2. Data Gathering Procedures

3.2.1. Preparation and Collection of Sample

Leaves of *J. gendarussa* were gathered from the province of eastern Samar, Philippines, for which taxonomic identification was done by an expert botanist.

3.2.2. Preparation of Leaf Extract

Using a scissor .veswere trimmed from its stem. Only young leaves were used.

The gathered leaves were soaked in n-hexane for 30 min and then pounded in a mortar and pestle. The extracted juice was then filtered using a Wattman No. 42 filter paper and a funnel, and then the filtrate was collected and put into a clean bottle.

3.3. Physical Property Analysis of Plant Extract

3.3.1. Boiling Point

About 5 mL of leaf extract of *J. gendarussa* was put in a test tube. The tube was then submerged in a water bath and the temperature where the extract started to boil was recorded. The process was repeated thrice.

3.3.2. Colour

Colour of the extract of *J. gendarussa* was determined using the sense of sight of a total of five respondents. About 10 mL of plant extract was contained into a clear Petri dish. Then, using the sense of sight, the respondents described the colour of the extract. Perception of colour from the respondents was gathered and analysed.

3.3.3. Density

To test for density, 10 mL of plant extract was weighed on an analytical balance in a pre-weighed graduated cylinder. The weight of the extract was then recorded and divided by the amount of extract used (in mL). The procedure was repeated thrice. Average density was also computed using the equation

3.3.4. Odour

Odour of the extract was tested using the olfactory sense of a total of five respondents. About 5 mL of the extract was contained into a clear transparent beaker. Then, the respondents described the odour of the extract contained in the transparent beaker. Perception of odour from the respondents was gathered and analysed.

3.3.5. pH

pH of the leaf extract was determined using a pH paper. About 5 mL of the extract was contained in a 50 mL beaker. Then, a pH paper was dipped into the extract. After 1 min, colour reaction of the pH paper was compared with the pH indicator, and then the pH was recorded. The procedure was repeated thrice. Average pH was also computed.

3.3.6. Miscibility

To test the miscibility of the plant extract, three solvents were used, namely benzene, water and ethanol. About 2 mL of the extract was put into nine clear test tubes. Then,

10 mL benzene was poured into three test tubes; another three test tubes were poured with 10 mL of water, and the remaining three test tubes with 10 mL ethanol. The nine test tubes were observed for 1 h to determine the miscibility of extract. The researchers recorded the finding as (1) miscible, (2) slightly miscible or (3) not miscible.

3.4. Percent Yield

Percent yield was computed using the formula

3.5. Screening Test for Active Components

Screening tests were performed for the confirmation of components including alkaloids, saponins, terpenoids, phenolic compounds, leucoanthocyanin, steroids, tannins and anthraquinones. **These substances were detected using the following procedures:**

3.5.1. Alkaloid

The extract of *J. gendarussa* was dissolved individually in diluted (2 M) hydrochloric acid and filtered, and then it was subjected to the following tests:

Mayer's test: The filtrates were treated with Mayer's reagent; the formation of a yellow coloured precipitate indicated the presence of alkaloids.

Dragendroff's test: The filtrates were treated with Dragendroff's reagent; the formation of red precipitate indicated the presence of alkaloids.

3.5.2. Anthraquinone

The Borntrager's test was used to determine the presence of anthraquinone. Red coloration in the lower ammoniacal layer indicated the presence of anthraquinone.

An equivalent of 10 g of extracted liquid from the stock plant extract was taken; it was evaporated to incipient dryness over a steam bath. Then to the residue 10 mL of distilled water was added and filtered. The aqueous filtrate was then extracted twice with a 5 mL portion of benzene, and combined with the benzene extract. Then the combined benzene extract was divided into two portions. One portion served as the control and other portion was treated with 5 mL ammonia solution and shaken. Then it was compared to the control tube.

3.5.3. Saponin

The capillary test was used to determine the presence of saponin. That is, if the level of the plant extract in the capillary tube is half than in the other tube containing water, the presence of saponin can be inferred.

This was done by loading a capillary tube with the leaf extract by immersing the tube to a height of 10 mm. in the plant extract. Likewise, another capillary tube was loaded with distilled water. Then the two tubes were kept in a vertical position to allow the liquid inside to flow out freely. After sometime, the heights of the liquids in the two tubes were compared.

3.5.4. Phenolic Compounds

The detection of phenols was carried out using the ferric chloride test. Leaf extracts were treated with 3–4 drops of ferric chloride solution. The formation of bluish black colour indicated the presence of phenols.

3.5.5. Tannins

The gelatin test was used to determine the presence of tannin in the leaf extract. To the leaf extract, 1% gelatin solution containing sodium chloride was added. The formation of white precipitate indicated the presence of tannins.

3.5.6. Steroid

The Libermann-Burchard test was used to detect the presence of terpenoid. A range of colours from blue to green, pink, violet or purple indicated the presence of terpenoid.

An equivalent amount of 10 g of extract from the prepared plant extract was evaporated to incipient dryness over a steam bath. It was then cooled to room temperature. Then the material was defatted by taking up residue with 12 mL hexane and 6 mL water. It was partitioned by gently shaking the mixture in a test tube. The upper hexane layer was pipetted out. Then, the treatment with hexane was repeated until most of the coloured pigments were removed. Hexane was discarded properly. The aqueous layer was then treated with 10 mL chloroform and the mixture was gently shaken. It was allowed to stand for a few minutes and the chloroform extract was again pipetted off. Then the chloroform extract was dried by filtering through about 100 mg of anhydrous sodium sulfate held over the dry Whattman 42 filter paper. The filtrate was then divided into two portions. One portion served as the control. Then the other portion was treated with three drops of acetic anhydride and then a drop of concentrated sulfuric acid. Any immediate colour change was observed. Then it was set aside for an hour

and observed for further colour changes. It was then compared with the control and the results were recorded.

3.5.7. Terpenoid

An equivalent amount of 10 g of extract from the prepared plant extract was evaporated to incipient dryness over a steam bath. It was then cooled to room temperature. Then the material was defatted by taking up residue with 12 mL hexane and 6 mL water. It was partitioned by gently shaking the mixture in a test tube. The upper hexane layer was pipetted out. Then, treatment with hexane was repeated until most of the coloured pigments were removed. Hexane was discarded properly. The aqueous layer was then treated with 10 mL chloroform and the mixture was gently shaken. It was allowed to stand for a few minutes and the chloroform extract was pipetted off. Then the chloroform extract was dried by filtering through about 100 mg of anhydrous sodium sulfate held over the dry filter paper. The filtrate was then divided into two portions. One portion served as the control. Then the other portion was treated with three drops of acetic anhydride and then a drop of concentrated sulfuric acid. Formations of blue-green rings indicated the presence of terpenoid in the extract.

4. Results

Results were recorded and summarised herein.

4.1. Physical Properties

Physical properties of the extract are reported in Table 1.

4.2. Percent Yield

By weighing the extracts *of J. gendarussa* and dividing it by its weight of leaves, percent yield was recorded to be at 30%. It was documented by collecting about 30 g of crude *J. gendarussa* extract from the total 100 g of leaves used in the conduct of this study.

Table 1. **Physical properties of *J. gendarussa* extract**

Physical properties	Observation
Boiling point	103.3°C
Colour	Brown
Odour	Pleasant odour
Density	1.02 g/mL
pH	7 (Neutral)
Miscibility	Miscible (in water)
	Miscible (in methanol)
	Immiscible (in chloroform)

Table 2. Phytochemical analysis

Secondary metabolites tested	Finding
Alkaloid	Positive
Anthraquinone	Negative
Leucoanthocyanin	Negative
Phenolic compounds	Negative
Saponin	Positive
Steroid	Negative
Tannin	Negative
Terpenoid	Negative

4.3. Phytochemical Screening

Based on the results of this study, only alkaloid and saponin are present in the plant extract of *J. gendarussa* leaves. Other secondary metabolites such as terpenoids, tannin, anthraquinone, leucoanthocyanin, phenolics, and steroids were found to be absent in the plant extract. These findings are summarised in Table 2.

5. Discussion

Justicia gendarussa, commonly known as willow-leaved Justicia, is a small, erect, branched shrub endemic to India. It is useful in the treatment of asthma, rheumatism and colics of children. It may have the potential to be used in male sterilization. Clinical tests are on-going in Indonesia.[4]

From the results of this study, *J. gendarussa* leaf extract was found to be brown in colour and pleasant in odour. Its boiling point is 103.3°C, with an average density of 1.02 g/mL, neutral pH of 7, and polar in compound constituent as evidenced by its miscibility in water and methanol and immiscibility in chloroform.

Further analysis revealed the presence of alkaloids and saponin only; these secondary metabolites were indicated by an orange and white precipitate for alkaloids and a strong capillary action for saponin comparison to distilled water. Other secondary metabolites were found to be negative in the plant extract, including tannin, phenolic compounds, terpenoids, steroids, anthraquinone and leucoanthocyanin; the absence of coloration and precipitates implied these secondary metabolites are absent in the plant extract.

6. Conclusions

Based on the findings of this study, the following conclusions were drawn:

1. *J. gendarussa* leaf extract has a high boiling point than water, a neutral pH of 7, and is polar in its chemical constituent.
2. *J. gendarussa* leaf extract has a percent yield of 30% per 100 g of leaves.
3. The leaf extract of *J. gendarussa* has the presence of alkaloid and saponin.
4. The leaf extract of *J. gendarussa* has no tannin, phenolic compounds, terpenoids, steroids, anthraquinone and leucoanthocyanin metabolites.

7. Recommendations

The following recommendations are drawn by the researcher in the direction of future work:

1. To characterise and quantify alkaloid and saponin in the extract of *J. gendarussa*.
2. To determine if there is a difference in secondary metabolite screening.
3. To make use of *J. gendarussa* as a natural source of alkaloid and saponin.

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