ULTRASOUND-ASSISTED EXTRACTION OF *ALSTONIA SCHOLARIS (L.) R. BR* STEM BARK: A PRELIMINARY PHYTOCHEMICAL CHARACTERIZATION

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ABSTRACT

Alstonia scholaris (L.) R. Br is a medicinal plant traditionally used for various therapeutic purposes and known to contain bioactive secondary metabolites. This study aimed to perform a preliminary phytochemical screening of the methanolic extract of A. scholaris stem bark obtained using ultrasound- assisted extraction (UAE). Dried stem bark collected from Central Borneo was extracted with methanol, yielding 5.29% (w/w) crude extract. Qualitative phytochemical screening was conducted using standard reagents to detect the presence of major secondary metabolite classes, including alkaloids, flavonoids, phenolics, saponins, and steroids/terpenoids. The results showed positive reactions for alkaloids and terpenoids, while flavonoids, phenolics, saponins, and steroids were not detected under the experimental conditions. This study provides preliminary phytochemical information on the methanolic stem bark extract of A. scholaris, which may serve as baseline data for further phytochemical and biological activity studies. These findings indicate that alkaloids and terpenoids are the dominant secondary metabolites in the methanol extract of *Alstonia scholaris (L.) R. Br* stem bark obtained by UAE, and provide a foundational profile for further studies on its biological activities.

Keywords: Alkaloids, *Alstonia scholaris*; Phytochemical screening; Pulai; Ultrasound-Assisted Extraction;

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INTRODUCTION

Indonesia is one of the world's mega biodiversity countries, with an exceptionally rich flora, including various medicinal plant species that have been used for generations in traditional medicine. One of these is Pulai or jelutung (*Alstonia scholaris* (L.) R.Br), also known locally as *pule* and *kayu gabus*. It is a hardwood tree native to Indonesia (especially Java and Sumatra) and widely utilized as a medicinal plant as well as for shade and reforestation purposes (Silalahi, 2019). Various parts of this plant, such as the leaves, bark, and latex, serve as examples of Indonesia's biodiversity resources used for the development of herbal medicines and health products (Zuraida et al., 2015).

Botanically, *Alstonia scholaris* is an evergreen tree with a straight trunk and gray to grayish-brown bark that exudes milky white latex when injured. It has whorled leaves, with 3–10 leaves per node, small yellowish-white flowers arranged in panicles at the ends of branches, and slender paired follicles containing many fine-haired seeds (Verma et al., 2024). The bark of the tree is

known to be bitter, and ethnomedicinally it has long been used by local communities as a traditional remedy. In several regions, decoctions of the bark are used to treat hypertension, diabetes, malaria, diarrhea, and hemorrhoids (Indartik, 2009; Bariyyah et al., 2025).

Phytochemical screening represents a crucial preliminary stage in medicinal plant research, as it enables the qualitative identification of major classes of secondary metabolites, such as alkaloids, flavonoids, phenolic compounds, terpenoids, steroids, and saponins. These compounds are frequently associated with diverse biological activities and serve as chemical markers for further isolation and bioactivity-guided studies (Tunny et al., 2022; Dhruti et al., 2016). Therefore, phytochemical screening provides initial chemical insight rather than direct evidence of therapeutic efficacy.

Previous studies have reported the presence of various secondary metabolites in the leaves and bark of A. scholaris. However, information regarding the phytochemical profile of methanolic extracts of the stem bark, particularly those obtained using ultrasound-assisted extraction, remains limited and fragmented. The choice of methanol as an extraction solvent is based on its effectiveness in extracting a broad range of polar and semi-polar secondary metabolites, while ultrasound-assisted extraction offers advantages in terms of extraction efficiency and reduced processing time.

MATERIALS AND METHODS

a. Materials

This research used several materials such as *Alstonia scholaris (L.) R. Br* powder, HCl 2N, Mayer's reagent, Liebermann - Burchard reagent, Dragendorf reagent, Mg powder, sulfuric acid, Acetic acid anhydrate, petroleum ether, FeCl3, Methanol, Aquadest, lab glassware, titer plate, and UAE (Ultrasound-Assisted Extraction) with below specification:

1. Model: GT SONIC-P3
2. Frequency: AC 220-240 V 50 Hz
3. Ultrasonic power: 30-100W
4. Heating power: 100W

b. Method

1. Plant collection and sortation
   The phytochemical screening method based on Maheswaran, 2024 with several modifications. Samples (*Alstonia scholaris*) are collected in Central. Sample dried with an oven method from 1500 gram fresh bark into 760 gram fine powder form.
2. Extraction
   Extraction method using Ultrasound-assisted Extraction, UAE has advantages such as less time and energy requirement, extraction at low temperature and retention of the quality of the extract (Kumar, 2021). Extraction process with methanol solvent to sample (5:1) around 5 minutes and triple re-maceration step. Crude extracts are obtained 81,6 mg with a yellowish black color identification.
3. Phytochemical screening
   Phytochemicals can be classified in various ways to better understand their diverse properties and potential health benefits. One common method is based on their chemical structure, dividing them into categories such as alkaloids, phenolics, terpenoids, glycosides, etc (Peiris, 2023). Qualitative phytochemical screening was conducted to identify the presence of major classes of secondary metabolites using standard chemical tests. All tests were performed using the methanolic extract under the same experimental conditions.

a) Alkaloids

The extracts was dissolved in dilute hydrochloric acid and filtered individually and tested for the presence of alkaloids. Mayers test: The extraction added to the Mayers reagent. A yellow cream precipitate formation indicates the presence of alkaloids (Nortjie, 2022).

The Dragendorf test principle is that the hydrolysis reaction regulates the presence of Bi3+ ions in the solution. The formed precipitate in excess potassium iodide dissolves to form potassium tetraiodobismuthate. Nitrogen forms a covalent coordination bond with the K+ ion (Parbuntari, 2018).

Bouchardat's reagent Made from a solution of iodine and potassium iodide. Iodine reacts with alkaloids, forming a colored precipitate. Iodine and alkaloids react, forming an insoluble complex. This produces a colored precipitate, indicating the presence of alkaloids (Maheswaran, 2024).

For alkaloid test, prepared sample adding 1 ml of HCl 2N and 4 ml of aquadest. Heat up until warm and then filtrate into a new solution. The solution then adds dragendorf, bouchardat, and mayer reagents.

b) Flavonoid

The Shinoda test for flavonoids is based on reducing flavonoids by metallic magnesium in the presence of hydrochloric acid, resulting in the formation of colored complexes. When magnesium is introduced to a solution containing flavonoids and concentrated hydrochloric acid is added, the flavonoid compounds undergo a reduction reaction. This reduction converts the flavonoids into their corresponding aglycone forms (non-glycosylated flavonoids). The interaction between the reduced flavonoids, magnesium, and hydrochloric acid leads to the development of distinctive colors, typically ranging from pink to red, that indicate the presence of flavonoids in the tested sample (Maheswaran, 2024). Profile of alkaloid test reagents presented in Table 1.

Table 1. Profile of alkaloid test reagents.

Reagent	Precipitation	Colour
Mayer	Positive	White/yellow
Bouchardats	Positive	Brown/black
Dragendorf	Positive	Dark red

c) Phenolic

The Ferric chloride reagent forms a colored complex between ferric ions (Fe^{3+}) and phenolic hydroxyl groups. The phenolic hydroxyl group reacts with ferric chloride to form a complex that exhibits a characteristic color change, depending on the specific phenol present in the sample (Aljamali, 2015).

d) Saponin

Saponins consist of polar groups, glycosyls, and steroids/triterpenoids as non-polar groups. Compounds containing both polar and nonpolar groups are surface active compounds. If these compounds are shaken vigorously with water, saponins will form miscellaneous. In the miscellaneous area, polar groups face out and non-polar groups face in. This phenomenon is known as foam (Parbuntari, 2018).

e) Steroid / Terpenoid

Based on the reaction between sterols and concentrated sulfuric acid. When cholesterol or other sterols are treated with concentrated sulfuric acid, they undergo sulfonation, forming a colored product. If a sample positive contains a terpenoid, the color changes into red, orange or yellow. If the sample positively contains steroids it turns into a green color (Maheswaran, 2024).

RESULT

Stem bark samples of Alstonia scholaris were collected from Central Borneo Province and used for extraction. Ultrasound-assisted extraction using methanol at a sample-to-solvent ratio of 1:5 (w/v) resulted in a crude

extract yield of 5.29% (w/w). Preliminary phytochemical screening was conducted to detect major classes of secondary metabolites. As summarized in Table 2, the methanolic stem bark extract tested positive for alkaloids and terpenoids, while steroids, flavonoids, and saponins were not detected under the experimental conditions.

The results showed that the methanol extract of *Alstonia scholaris* was positive for alkaloids and terpenoid. This finding is consistent with the study conducted by (Verma et al., 2025) which reported that alkaloids are the major constituents of this species

Table 2. Phytochemical Screening of *Alstonia scholaris* methanol extract

Secondary Metabolite	Result
Alkaloid	+
Terpenoid	+
Steroid	-
Flavonoid	-
Saponin	-

DISCUSSION

The extraction was carried out using the Ultrasonic-assisted Extraction (UAE) method. The UAE technique not only provides substantial benefits in enhancing the yield of bioactive compounds, but it also produces various positive or negative impacts on the physicochemical characteristics related to their bioactivity (Shen et al., 2023). UAE employs thermal, mechanical, and cavitation effects to facilitate the extraction of bioactive components. Under ultrasonic treatment, the cell wall is disrupted, which in turn accelerates the release and diffusion of intracellular constituents (Chemat et al., 2017).

Based on the study conducted by (Hayati et al., 2019) the extraction of *Alstonia scholaris* using methanol as solvent and the UAE method had an optimal extraction time of 10 minutes, as indicated by optimal spot intensity on TLC. The extraction was performed using the UAE method at room temperature with a simplicia to solvent ratio of 1:5. The UAE method was combined with three cycles of remaceration, with the rationale that after the first maceration, a portion of the bioactive compounds remains in the residue. By performing remaceration (re-soaking with fresh solvent), the remaining unextracted compounds can be further recovered, thereby increasing the overall yield of the extract. The yield of extracts obtained from the steam bark of the plants was founds to be 5.29% w/w.

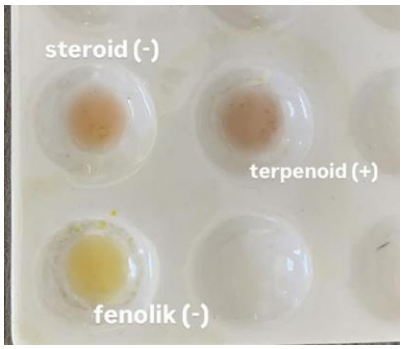

Figure 1. Phytochemical screening results of the methanol extract of *Alstonia scholaris* for steroids, terpenoid and phenolic

Based on previous study by Sumah et al, 2012, which stated that *Alstonia scholaris* contains alkaloids that are commonly isolated from higher plants and possess antiprotozoal activity.

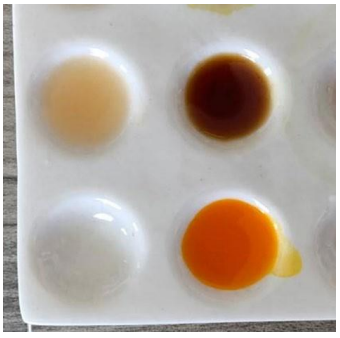

Figure 2. Screening results of the methanol extract of *Alstonia scholaris* for alkaloid

Alstonia scholaris bark extract is known to contain flavonoids, alkaloids, saponins, tannins, and terpenoids (Saxena et al., 2013). Administration of the methanol extract of *Alstonia scholaris* stem bark was shown to affect leukocyte differentiation in mice infected with Plasmodium berghei ANKA by increasing neutrophil and monocyte counts and decreasing eosinophil and lymphocyte counts toward normal ranges. Among the tested doses, 200 mg/kg body weight produced the most optimal effect on the improvement of leukocyte profiles (Mayor & Wattimena, 2022). In Indonesia, *Alstonia scholaris* are often found and easily available in Kalimantan (Dwi Agustina et al., 2023). This is in line with the findings of (Mayor & Wattimena, 2022) reporting that *Alstonia scholaris* stem bark is used by the local community in Raja Ampat Regency for the treatment of malaria.

Methanol as a polar-protic solvent effectively extracts alkaloids because it can interact with weakly basic groups through hydrogen bonding, while the Ultrasonic Assisted Extraction (UAE) method further enhances extraction efficiency through cavitation effects that disrupt cell walls and accelerate the release of bioactive compounds. The absence of flavonoids, steroids, and saponins is likely due to differences in solvent polarity, the sensitivity of certain compounds to ultrasonic waves, geographical variation of the plant, and the plant part used. Overall, the combination of methanol and UAE in this study successfully isolated alkaloids optimally and produced a phytochemical profile consistent with the chemical characteristics of *Alstonia scholaris* (Akinmoladun et al., 2022).

The phytochemical screening of the methanol extract of *Alstonia scholaris* still has several limitations, such as the absence of metabolite profile confirmation using advanced analytical methods, the lack of solvent variation to explore a broader spectrum of metabolites, and the absence of detailed optimization of UAE parameters. Future studies should consider the use of gradient solvents, validation of metabolite content with modern chromatographic instruments, specific biological activity testing, and optimization of UAE conditions to obtain a more comprehensive phytochemical profile and support the utilization of *Alstonia scholaris* as a source of bioactive compounds.

CONCLUSION

Samples are collected in Central Borneo province. Dried sample with an oven method from 1500 gram fresh bark into 760 gram fine powder form. Crude extracts are obtained 81,6 mg with a yellowish black color identification. From the phytochemical screening result, it can be concluded that crude extract positively consists of Alkaloid dan Terpenoid.

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