

Phytochemical Screening, Antioxidant Activity, And Antimitotic Potential Of Shallot Skin (*Allium Cepa* L.) Extract

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ABSTRAK

Shallot skin (*Allium cepa* L.) is a by-product that is generally discarded as household waste, despite its potential as a source of bioactive compounds. Previous studies have indicated that shallot skin contains secondary metabolites with antioxidant and cytotoxic properties. This study aimed to evaluate the phytochemical composition, antioxidant activity, and antimitotic potential of shallot skin extract in order to support its valorization as a natural bioactive resource.

Materials and methods. This research was conducted as an experimental laboratory study. Dried shallot skin was extracted using ethanol through a maceration method, followed by solvent evaporation to obtain a crude extract. Phytochemical screening was performed using Thin Layer Chromatography (TLC) to identify major secondary metabolites. Solvent partitioning was carried out using n-hexane and ethyl acetate, and further fractionation was conducted using vacuum column chromatography and preparative TLC. Antioxidant activity was evaluated using the DPPH radical scavenging assay, while antimitotic activity was assessed using the Brine Shrimp Lethality Test (BSLT).

Results. The ethanolic extraction produced a high yield of crude extract (57.13%), indicating effective recovery of polar and semi-polar compounds. Phytochemical screening confirmed the presence of alkaloids, flavonoids, and phenolic compounds. The extract exhibited very strong antioxidant activity with an IC₅₀ value of 17.477 µg/mL. Antimitotic evaluation using BSLT showed an LC₅₀ value of 202.301 µg/mL, indicating biological activity at moderate concentrations.

Conclusions. The findings demonstrate that shallot skin contains diverse secondary metabolites with strong antioxidant activity and measurable antimitotic potential. These results support the utilization of shallot skin as a valuable natural source of bioactive compounds and provide a basis for further studies on compound isolation and advanced biological evaluation.

Keywords: Shallot skin; Antioxidant activity; DPPH; Brine Shrimp Lethality Test; Phytochemical screening

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INTRODUCTION

Shallot (*Allium cepa* L.) is one of the most widely cultivated horticultural crops and plays a crucial role as a culinary spice and natural flavor enhancer. Its utilization is predominantly focused on the bulb, which is valued for its sensory, nutritional, and medicinal properties. In contrast, the outer dry skin of shallot is generally discarded and treated as household waste,

despite increasing evidence that it contains high-value bioactive compounds with significant pharmacological potential (Suwardi & Noer, 2020; Suryandari & Kusumo, 2022).

The continuous increase in shallot consumption contributes to the accumulation of shallot skin waste from households, traditional markets, and food-processing industries. Limited utilization of this by-product not only adds to environmental burden but also represents a loss of potential natural resources. Recent studies have reported that shallot skin is rich in secondary metabolites, including flavonoids, phenolic compounds, saponins, alkaloids, terpenoids, and steroids, which are known to exhibit strong antioxidant activity (Mardiah et al., 2017; Suryandari & Kusumo, 2022).

Among these compounds, flavonoids particularly quercetin and its derivatives are recognized as major contributors to antioxidant activity. Antioxidants play a vital role in scavenging free radicals and reducing oxidative stress, which is closely associated with the development of degenerative diseases such as cardiovascular disorders, diabetes mellitus, cancer, and atherosclerosis. Several recent studies have demonstrated that ethanolic extracts and fractions of shallot skin show strong radical scavenging activity when evaluated using the DPPH method, indicating their potential as natural antioxidant sources (Martha, 2019; Suwardi & Noer, 2020).

In addition to antioxidant properties, natural product extracts are frequently evaluated for their cytotoxic and antimitotic potential as part of early-stage screening for anticancer activity. The Brine Shrimp Lethality Test (BSLT) is widely used as a preliminary bioassay due to its simplicity, low cost, and strong correlation with cytotoxic activity. Extracts exhibiting significant lethality toward *Artemia salina* larvae are considered to possess biologically active compounds that may have further pharmacological relevance (Jelita et al., 2020; Kurniawan & Ropiqa, 2021). Despite growing interest in the biological activities of shallot skin, studies that integrate phytochemical screening, chromatographic isolation, antioxidant evaluation, and toxicity assessment remain limited, particularly within the last five years. Many investigations focus solely on crude extracts without comprehensive fractionation and purification to better associate specific chemical constituents with their biological activities. Therefore, an integrated approach combining extraction, partitioning, chromatographic separation, antioxidant activity testing using the DPPH method, and toxicity evaluation through BSLT is essential to provide a more comprehensive understanding of the bioactive potential of shallot skin (Aziz et al., 2019; Suryandari & Kusumo, 2022).

This study aims to analyze the phytochemical constituents of shallot skin (*Allium cepa* L.) through systematic extraction, partitioning, and chromatographic isolation, as well as to evaluate its antioxidant activity using the DPPH radical scavenging method and its antimitotic potential using the Brine Shrimp Lethality Test. The findings of this study are expected to support the utilization of shallot skin waste as a valuable natural antioxidant and bioactive resource, contributing to the development of natural products in pharmaceutical and functional food applications.

METHOD

Study Design

This research was an experimental laboratory study conducted to evaluate the phytochemical constituents, antioxidant activity, and antimitotic potential of shallot skin (*Allium cepa* L.). The study involved extraction, solvent partitioning, chromatographic isolation, antioxidant activity testing using the DPPH method, and antimitotic activity evaluation using the Brine Shrimp Lethality Test (BSLT).

Materials

The plant material used in this study was shallot skin (*Allium cepa* L.) obtained from household waste. The chemicals used were of analytical grade, including 96% ethanol, n-hexane, ethyl acetate, methanol (p.a.), chloroform, distilled water, and DPPH solution. *Artemia salina* Leach eggs and natural seawater were used for the antimitotic assay.

Instruments

The instruments used in this study included glass funnels, measuring cylinders, dropper pipettes, filter cloths, beakers, separating funnels, rotary vacuum evaporator, round-bottom flasks, chromatographic columns, silica gel (TLC F254), UV lamps (254 nm and 366 nm), analytical balance, micropipettes, centrifuge, aerator, and spectrophotometers (UV-Vis, IR, MS, and NMR).

Extraction Procedure

The dried shallot skin was weighed and placed into a glass container. The material was completely immersed in 96% ethanol and stirred evenly. The container was sealed and left to macerate for 72 hours at room temperature with occasional stirring every 24 hours. After maceration, the mixture was filtered, and the filtrate was collected. The solvent was removed using a rotary vacuum evaporator to obtain a concentrated crude extract.

Solvent Evaporation

The liquid extract was transferred into a round-bottom flask filled to approximately two-thirds of its volume and connected to a rotary vacuum evaporator. The water bath temperature was set at 5–10°C below the boiling point of the solvent. Vacuum pressure and rotation were applied until the solvent was completely evaporated, yielding a viscous extract.

Phytochemical Screening by Thin Layer Chromatography (TLC)

Thin Layer Chromatography was performed to identify secondary metabolite groups. The TLC chamber was saturated with a mixture of n-hexane and ethyl acetate as the mobile phase. The extract was spotted onto silica gel TLC plates using capillary tubes and allowed to dry. Plates were developed until the solvent front reached the upper limit. The chromatograms were observed under UV light at 254 nm and 366 nm. Detection reagents were used to identify compound groups: Dragendorff reagent for alkaloids, citroborate reagent for flavonoids, and ferric chloride (FeCl_3) reagent for phenolic compounds.

Solvent Partitioning

Approximately 1–2 g of crude extract was suspended in 20 mL of distilled water and transferred into a separating funnel. The suspension was partitioned with 40 mL of n-hexane, shaken thoroughly, and allowed to separate into two phases. The aqueous phase was re-extracted three times. The remaining aqueous fraction was then partitioned with ethyl acetate using the same procedure. Each fraction was collected and evaporated to obtain the respective extracts.

Isolation of Natural Compounds

Isolation was carried out using Vacuum Column Chromatography (VCC). Silica gel 60 was used as the stationary phase, consisting of 40 g coarse silica gel and 10 g fine silica gel. Approximately 1 g of the ethyl acetate fraction was applied to the column. Elution was performed using n-hexane and ethyl acetate with gradually increasing polarity ratios (10:0 to 0:10). A total of thirteen fractions were collected and analyzed using TLC.

Fractionation and Purification

Further purification was conducted using Preparative Thin Layer Chromatography (PTLC). The extract was applied as a band on the TLC plate and developed with an appropriate solvent system. Visible bands were marked, scraped, and eluted using methanol. Additional analysis was performed using multi-eluent TLC and two-dimensional TLC to confirm compound purity. Retardation factor (R_f) values were calculated for each detected spot.

Antioxidant Activity Assay (DPPH Method)

A total of 10 mg of extract was dissolved in methanol and diluted to obtain concentrations of 20, 40, 60, 80, and 100 ppm. Each sample solution (0.5 mL) was mixed with 3.5 mL of DPPH solution and incubated in the dark for 30 minutes. Absorbance was measured using a UV-Vis

spectrophotometer. The percentage of radical scavenging activity was calculated, and the IC₅₀ value was determined from the linear regression analysis.

Antimitotic Activity Assay (Brine Shrimp Lethality Test)

Artemia salina eggs were hatched in seawater under continuous aeration and illumination for 48 hours. Test solutions were prepared at concentrations of 90 ppm and 120 ppm. Ten larvae aged 48 hours were transferred into vials containing 5 mL of test solution. Each concentration was tested in triplicate along with a control group. After 24 hours of incubation, larval mortality was recorded, and the LC₅₀ value was calculated.

Data Analysis

Antioxidant activity was expressed as IC₅₀ values, while antimitotic activity was expressed as LC₅₀ values. TLC results were analyzed qualitatively based on spot appearance and R_f values. Data were presented descriptively and analytically.

FINDING AND DISCUSSION

Table 1. Extraction Yield of Shallot Skin (*Allium cepa* L.)

Parameter	Value
Weight of dried simplicia (g)	60.00
Weight of crude extract (g)	34.28
Extraction yield (%)	57.13

Table 2. Phytochemical Screening Results by Thin Layer Chromatography (TLC)

Parameter	Value
Alkaloids (Dragendorff)	Brown precipitate/spot – Positive (+)
Flavonoids (Citroborate)	Yellow fluorescence – Positive (+)
Phenolic compounds (FeCl ₃)	Black coloration – Positive (+)

Table 3. Solvent Partitioning Yield of Shallot Skin Extract

Parameter	Value
n-Hexane fraction (g / %)	10.00 / 66.66
Ethyl acetate fraction (g / %)	5.00 / 33.33
Total (g / %)	15.00 / 100.00

Table 4. Fractionation Results by Vacuum Column Chromatography (VCC)

Parameter	Value
Stationary phase	Silica gel 60
Mobile phase	n-Hexane : Ethyl acetate (10:0 □ 0:10)
Number of fractions	13
Fraction color variation	Clear to yellowish

Table 5. Retardation Factor (R_f) Values from TLC Analysis

Parameter	Value
Preparative TLC (PTLC)	0.32
Two-dimensional TLC	0.23

Table 6. Antioxidant Activity of Shallot Skin Extract (DPPH Assay)

Parameter	Value
Regression equation	$y = -0.0028x + 1.062$
RI value	0.9926
IC ₅₀ (µg/mL)	17.477
Antioxidant strength	Very strong

Table 7. Antimitotic Activity of Shallot Skin Extract (BSLT Assay)

Parameter	Value
Test organism	Artemia salina larvae
Exposure time (hours)	24
Number of larvae per vial	10
LC ₅₀ (µg/mL)	202.301
Toxicity category	Toxic

Extraction yield was calculated as the percentage ratio between the weight of crude extract and dried simplicia. Phytochemical screening results are reported qualitatively as positive (+) based on characteristic color reactions and fluorescence observed on TLC plates under UV light (254 and 366 nm). Solvent partitioning percentages represent the proportion of each fraction relative to the total partitioned extract weight. Rf values were calculated as the ratio of the distance traveled by the compound to the distance traveled by the solvent front. Antioxidant activity was evaluated using the DPPH radical scavenging assay, and IC₅₀ values were obtained from linear regression analysis of absorbance versus concentration, where lower IC₅₀ values indicate stronger antioxidant activity. Antimitotic activity was assessed using the Brine Shrimp Lethality Test (BSLT), and LC₅₀ values represent the concentration required to cause 50% mortality of *Artemia salina* larvae after 24 hours of exposure. Toxicity classification was determined based on standard LC₅₀ thresholds for preliminary cytotoxicity screening.

Discussion

The high extraction yield obtained in this study indicates that ethanol maceration is an effective method for recovering bioactive compounds from shallot skin. Ethanol is capable of extracting a wide range of polar and semi-polar constituents, which is consistent with the chemical nature of secondary metabolites commonly found in plant-based materials. Similar findings have been reported in recent studies demonstrating that ethanolic extraction effectively isolates flavonoids and phenolic compounds from plant waste materials (Suwardi & Noer, 2020; Suryandari & Kusumo, 2022). The substantial yield also reflects the rich phytochemical content of shallot skin, supporting its potential valorization as a functional natural resource rather than household waste.

Phytochemical screening confirmed the presence of alkaloids, flavonoids, and phenolic compounds in the extract. These metabolite groups are well recognized for their antioxidant and cytotoxic properties. Recent studies have shown that flavonoids and phenolic compounds play a major role in scavenging free radicals and inhibiting oxidative stress through hydrogen or electron donation mechanisms (Pratama & Busman, 2020; Suryandari & Kusumo, 2022). The detection of these compounds provides a strong chemical basis for the antioxidant activity observed in this study.

Solvent partitioning showed that the majority of bioactive compounds were associated with the ethyl acetate fraction, indicating a predominance of semi-polar constituents. Although the n-hexane fraction accounted for a larger mass, chromatographic behavior and visual observations suggested limited biological relevance of non-polar components. This pattern is consistent with previous reports indicating that flavonoids and phenolic compounds are preferentially enriched

in semi-polar solvent fractions such as ethyl acetate (Suwardi & Noer, 2020; Suryandari & Kusumo, 2022).

Chromatographic isolation using vacuum column chromatography successfully separated the extract into multiple fractions, demonstrating the chemical complexity of shallot skin. The gradual color changes observed during gradient elution indicate effective polarity-based separation of compounds. Similar chromatographic profiles have been reported in natural product isolation studies, where gradient elution enhances the resolution of compounds with varying polarity (Aziz et al., 2019; Suryandari & Kusumo, 2022). Subsequent purification using preparative and two-dimensional TLC confirmed the presence of discrete compounds and supported the reliability of the fractionation process.

The antioxidant activity of the extract was classified as very strong based on the low IC_{50} value obtained from the DPPH assay. This result highlights the significant radical scavenging capacity of shallot skin extract and reinforces the role of flavonoids and phenolic compounds as the primary contributors to antioxidant activity. Comparable antioxidant potency has been reported in recent studies evaluating plant-derived waste materials using the DPPH method (Suwardi & Noer, 2020; Pratama & Busman, 2020). The strong antioxidant potential suggests possible applications in the development of natural antioxidant agents.

The Brine Shrimp Lethality Test demonstrated that the extract exhibited toxic effects at moderate concentrations, as reflected by the LC_{50} value. This finding indicates the presence of biologically active compounds with antimitotic or cytotoxic potential. Previous studies have shown that plant extracts exhibiting LC_{50} values below 1000 $\mu\text{g/mL}$ in BSLT assays are considered biologically active and warrant further cytotoxic evaluation (Jelita et al., 2020; Kurniawan & Ropiqa, 2021). While BSLT is a preliminary screening method, the observed toxicity supports further investigation using targeted cytotoxicity assays to evaluate potential anticancer activity.

In summary, the results demonstrate that shallot skin contains diverse secondary metabolites with strong antioxidant activity and measurable antimitotic potential. These findings are consistent with recent literature highlighting the pharmacological value of plant-based waste materials and support the utilization of shallot skin as a valuable source of bioactive compounds. Furthermore, the results provide a foundation for future studies focusing on compound isolation, structural characterization, and advanced biological evaluation (Suwardi & Noer, 2020; Suryandari & Kusumo, 2022).

CONCLUSIONS

This study aims to see the extent of the influence of organizational culture and work life balance on employee performance with job satisfaction as an intervening variable. Based on the results of research and hypothesis testing that has been carried out, the results of the study can be concluded that organizational culture has a positive and significant effect on job satisfaction at the Agam Regency Land Office. Work life balance has a positive and significant effect on job satisfaction at the Agam Regency Land Office. Organizational culture has a positive and significant effect on employee performance at the Agam Regency Land Office. Work life balance has no significant effect on employee performance at the Agam Regency Land Office. Job satisfaction has a positive and significant effect on employee performance at the Agam Regency Land Office. Organizational culture has a positive and significant effect on employee performance through job satisfaction as intervening at the Agam Regency Land Office. Work life balance has a positive and significant effect on employee performance through job satisfaction as intervening at the Agam Regency Land Office.

It is hoped that the Agam Regency Land Office will consider organizational culture and work life balance as one of the indicators in increasing job satisfaction, because organizational culture and work life balance have a significant effect on job satisfaction. The better and more adequate the organizational culture and the higher the work life balance, the more job satisfaction and employee performance will increase. The results of this study are expected to add insight, knowledge, and experience as well as a provision

in applying the knowledge that has been obtained in college in knowing the extent of the relationship between the theory obtained in lectures and the real conditions that exist in the field. For further researchers, it is expected to develop the results of this study and involve relevant variables related to organizational culture and work life balance on employee performance mediated by job satisfaction. To facilitate research, future researchers can use a link questionnaire for a wider range of questionnaire distribution such as using google form, in the hope that the research results are more accurate and have far greater benefits.

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