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Analysis Antioxidant of Clove Leaves (*Syzygium aromaticum*) as a Source of Bioactive Compounds

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ABSTRACT

This study aimed to evaluate the antioxidant activity of the chloroform extract of clove leaves (*Syzygium aromaticum*) as a source of bioactive compounds. Unlike most previous studies that predominantly focused on essential oils or polar extracts of clove, this research specifically investigates the antioxidant potential of the semi-polar chloroform extract, which remains relatively underexplored. Extraction was performed using chloroform to obtain semi-polar compounds with potential biological activity. Phytochemical screening revealed the presence of phenolic compounds, flavonoids, tannins, and terpenoids, which are known to function as natural antioxidants. Antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay based on the ability of the sample to neutralize free radicals. The results demonstrated an increase in percentage inhibition with increasing extract concentration, indicating a positive linear relationship between concentration and antioxidant activity. Linear regression analysis yielded an IC_{50} value of 69.40 ppm, classifying the extract as having strong antioxidant activity. In comparison, ascorbic acid exhibited an IC_{50} value of 8.75 ppm, indicating very strong antioxidant activity. This difference is attributed to the nature of ascorbic acid as a pure compound, whereas the chloroform extract consists of a complex mixture of secondary metabolites. The novelty of this study lies in highlighting the significant antioxidant potential of the semi-polar chloroform fraction of clove leaves, thereby expanding the understanding of solvent-specific bioactive compound distribution and providing new insight into its possible application in functional food and pharmaceutical development. Overall, the findings suggest that the chloroform extract of clove leaves has promising potential as a natural source of bioactive compounds for further development.

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INTRODUCTION

Free radicals are molecules or atoms that possess one or more unpaired electrons, making them highly reactive. The presence of free radicals in the body can trigger oxidative stress, which contributes to various degenerative diseases such as cancer, diabetes mellitus, cardiovascular diseases, and premature aging (Valko et al., 2007). To counteract the negative effects of free radicals, the body requires antioxidant compounds capable of donating electrons to stabilize these reactive species (Halliwell & Gutteridge, 2015). With increasing public awareness of food safety and health, the search for natural antioxidant sources has intensified as alternatives to synthetic antioxidants that may cause adverse side effects (Shahidi & Ambigaipalan, 2015). Oxidative stress occurs when the production of free radicals exceeds the capacity of endogenous antioxidant defense systems, leading to damage of essential cellular components such as lipids, proteins, and DNA, thereby disrupting normal physiological functions (Pham-Huy et al., 2008). Therefore, the intake of exogenous antioxidants is crucial to maintain redox balance and prevent cellular damage (Wael, 2018). Natural antioxidants derived from plants generally contain phenolic and flavonoid compounds that act as radical scavengers. Their mechanism of action involves hydrogen atom or electron donation and stabilization of radicals through resonance of their chemical structures (Prior et al., 2005). Compared to synthetic antioxidants, natural antioxidants exhibit lower toxicity and may provide additional biological activities, including anti-inflammatory and antimicrobial effects (Shahidi & Ambigaipalan, 2015).

Indonesia, as a tropical country, possesses exceptionally high biodiversity and great potential as a source of bioactive compounds (Wael, 2019). The utilization of local plants as natural antioxidant sources represents a strategic approach to support the development of pharmaceutical products, functional foods, and natural-based cosmetics, in line with environmentally friendly green industry initiatives (Atanasov et al., 2021). In the context of sustainability, the valorization of agricultural waste as a source of bioactive compounds is highly relevant. Previously underutilized waste materials can be processed into value-added products through extraction and characterization of active compounds, thereby reducing environmental impact and improving resource efficiency (Ayala-Zavala et al., 2011). Clove (*Syzygium aromaticum*) is one of Indonesia's important plantation commodities rich in bioactive compounds. Recent studies have reported that distilled clove leaf waste contains high levels of phenolics, flavonoids, and tannins with significant antioxidant activity (Mamahit et al., 2025). Chemical analyses of clove leaf and flower essential oils have also demonstrated strong antioxidant activity, with eugenol identified as the primary component responsible for radical scavenging activity (Muderawan et al., 2025). Furthermore, hydrothermal extraction of clove leaf waste has been shown to increase phenolic content and enhance DPPH and FRAP activities, indicating high antioxidant potential (Muaja et al., 2025). Other studies employing modern extraction techniques such as supercritical fluid extraction have reported significant levels of antioxidant compounds and eugenol in clove leaf extracts, demonstrating strong antioxidant activity (2023).

Extraction is a critical step in isolating bioactive compounds from natural materials. The choice of solvent polarity significantly influences the type and quantity of compounds extracted; therefore, selecting an appropriate solvent and extraction method is essential to target specific antioxidant compounds (Dai & Mumper, 2010). Chloroform, a semi-polar solvent, has the potential to extract certain bioactive compounds that are less soluble in polar solvents. Based on this background, this study aimed to evaluate the antioxidant potential of the chloroform extract of clove leaves (*Syzygium aromaticum*) as a source of bioactive compounds. The findings are expected to provide scientific information regarding the utilization of clove leaves and to support the development of natural-based products with antioxidant properties.

MATERIALS AND METHOD

Clove leaves (*Syzygium aromaticum*) were obtained from a clove plantation in Negeri Lima Village, Leihitu District, Indonesia. Fresh leaves were collected directly from the trees. The chemicals used in this study included absolute chloroform, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid (positive control), iron(III) chloride ($FeCl_3$), magnesium powder, concentrated hydrochloric acid, Liebermann–Burchard reagent, and distilled water. All chemicals were of analytical grade. The equipment used included an analytical balance, drying oven, blender, 60-mesh sieve, beakers, Erlenmeyer flasks, volumetric flasks, Whatman No. 1 filter paper, rotary evaporator, water bath, vortex mixer, micropipettes, and a UV–Vis spectrophotometer.

Sample Preparation. Clove leaves were washed under running water to remove impurities and then dried in an oven at 40–50°C until constant weight was achieved. The dried samples were ground using a blender and sieved through a 60-mesh sieve to obtain a uniform particle size. The powdered simplicia was stored in a sealed container at room temperature until extraction.

Extraction Procedure. A total of 500 g of clove leaf powder was macerated with chloroform at a ratio of 1:10 (w/v). The maceration process was carried out for three days at the Basic Biology Laboratory, Universitas Pattimura, at room temperature with occasional stirring. The filtrate was separated using Whatman No. 1 filter paper, and the residue was re-macerated twice with fresh solvent to ensure optimal extraction. All filtrates were combined and concentrated using a rotary evaporator at 40°C to obtain a viscous extract. The extract was weighed to calculate extraction yield and stored in a dark bottle at 4°C until further analysis. Extraction yield was calculated using the following formula: Yield (%) = (weight of extract / initial weight of simplicia) × 100%.

Phytochemical Screening. Qualitative phytochemical analysis of the chloroform extract was performed to detect the presence of secondary metabolites. Phenolic compounds were identified by the addition of 1% $FeCl_3$ solution, indicated by the formation of a dark green coloration. Flavonoids were tested using the magnesium–HCl method, indicated by the appearance of red or orange coloration. Terpenoids and steroids were detected using Liebermann–Burchard reagent, indicated by green or blue color changes. The presence of tannins was confirmed by the formation of a dark coloration after $FeCl_3$ addition.

Antioxidant Activity Assay (DPPH Method). The antioxidant activity of the chloroform extract was evaluated using the DPPH radical scavenging method. A 0.1 mM DPPH solution was prepared in methanol and stored in the dark. The extract was prepared at various concentrations (25, 50, 75, 100, and 125 ppm). One milliliter of extract solution was mixed with 1 mL of DPPH solution and incubated in the dark at room temperature for 30 minutes. Absorbance was measured using a UV–Vis spectrophotometer at 517 nm. Ascorbic acid was used as a positive control under the same procedure. The percentage of radical inhibition was calculated using the following equation: % Inhibition = [(A_control − A_sample) / A_control] × 100%. The IC_{50} value was determined by linear regression analysis between sample concentration and percentage inhibition.

Data Analysis. All experiments were performed in triplicate. Data were expressed as mean ± standard deviation. Linear regression analysis was conducted using Microsoft Excel to determine the IC_{50} value. Antioxidant activity was categorized based on IC_{50} values, where lower IC_{50} values indicate stronger antioxidant activity.

RESULTS AND DISCUSSION

Maceration of 500 g of clove leaf (*Syzygium aromaticum*) powder using chloroform produced a viscous greenish-brown extract with a characteristic clove aroma. The extract obtained weighed 30.5 g, corresponding to a yield of 6.50%. This yield indicates that chloroform effectively extracted semi-polar compounds present in clove leaves. Extraction yield is influenced by solvent type, extraction duration, particle size, and the secondary metabolite content of the plant material. Due to its semi-polar nature, chloroform preferentially extracts compounds such as terpenoids, certain flavonoid aglycones, and essential oil components (e.g., eugenol), rather than highly polar compounds.

Phytochemical Screening. The results of phytochemical screening of the chloroform extract of clove leaves are presented in **Table 1.**

Table 1. Phytochemical Screening Results of Chloroform Extract of Clove Leaves

Metabolite Compound		
Phenolics	+	Formation of dark green coloration
Flavonoids	+	Formation of reddish-orange color
Tannins	+	Dark green coloration
Terpenoids	+	Bluish-green coloration
Steroids	−	No significant color change

Note: (+) detected, (−) not detected.

The results indicate that the chloroform extract contains phenolics, flavonoids, tannins, and terpenoids. The presence of these compounds contributes to antioxidant activity due to the hydroxyl groups in phenolics and flavonoids, which can donate hydrogen atoms or electrons to neutralize free radicals. This mechanism has been widely reported as the fundamental basis of antioxidant activity in phenolic and flavonoid compounds (Shahidi & Ambigaipalan, 2015; Pietta, 2000). Tannins, as part of the polyphenol group, also exhibit free radical scavenging and metal chelating activities, which inhibit oxidative reactions (Hagerman et al., 1998). Detected terpenoids potentially contribute to antioxidant activity, especially if they contain phenolic structures such as eugenol, the main component of *Syzygium aromaticum*. Eugenol is known to exhibit significant antioxidant activity through hydrogen donation and radical stabilization mechanisms (Gulcin, 2011).

Antioxidant Activity (DPPH Assay). The antioxidant activity of the chloroform extract of clove leaves was evaluated using the DPPH method at 517 nm. The percentage inhibition at various concentrations is presented in **Table 2.**

Table 2. Antioxidant Activity of Chloroform Extract of Clove Leaves

Concentration (ppm)	% Inhibition (Mean ± SD)
25	22.39 ± 0.80
50	39.77 ± 1.13
75	57.13 ± 0.97
100	75.48 ± 1.01
125	89.90 ± 0.83

An increase in percentage inhibition was observed with increasing extract concentration, indicating a direct proportional relationship between extract concentration and DPPH radical scavenging ability. The DPPH assay (2,2-diphenyl-1-picrylhydrazyl) is widely used to evaluate antioxidant activity based on the ability of compounds to donate hydrogen atoms or electrons to reduce DPPH radicals (Blois, 1958; Brand-Williams et al., 1995). Linear regression analysis between concentration and percentage inhibition yielded an IC_{50} value of 69.40 ppm, categorizing the chloroform extract's antioxidant activity as strong (IC_{50} between 50-100 ppm). The classification of antioxidant strength based on IC_{50} values is commonly applied in phytochemical studies: IC_{50} < 50 ppm is very strong, 50-100 ppm strong, 100–150 ppm moderate, and >150 ppm weak (Molyneux, 2004). Ascorbic acid was used as a reference, showing an IC_{50} value of 8.75 ppm, classified as very strong. The higher IC_{50} of the extract is expected since ascorbic acid is a pure compound, whereas the chloroform extract is a complex mixture of secondary metabolites that may exert synergistic or antagonistic effects on antioxidant activity (Shahidi & Ambigaipalan, 2015).

The antioxidant activity observed is primarily attributed to phenolic and flavonoid compounds detected via phytochemical screening (Wansi et al., 2018). These compounds possess hydroxyl groups capable of donating electrons or hydrogen atoms to neutralize free radicals, rendering them more stable and non-reactive. Phenolics and flavonoids act through hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms, effectively reducing DPPH radicals to non-radical forms, accompanied by a color change from purple to yellow (Prior, Wu, & Schaich, 2005). The use of chloroform as a semi-polar solvent in this study facilitates the extraction of semi-polar compounds such as eugenol and other phenylpropanoid derivatives that are less soluble in polar solvents. Eugenol has been reported as a major constituent of clove leaf extracts with significant antioxidant activity in various in vitro assays (Shende & Shilpashree, 2024). This indicates that although not all highly polar phenolics are extracted, the semi-polar fraction still contains biologically relevant bioactive compounds.

The IC_{50} value of 69.40 ppm confirms that the chloroform extract of clove leaf waste exhibits strong antioxidant activity according to DPPH assay criteria. Although this value is higher than that of the ascorbic acid standard, such differences are reasonable given the complex nature of the extract compared to a pure standard compound (Nainggolan, Rahayu, & Rejeki, 2024). Therefore, the chloroform extract shows promise as a natural antioxidant source, albeit not as potent as polar fractions (Nusaly et al., 2023). The presence of phenolic compounds is consistent with other studies demonstrating a positive correlation between total phenolic content and free radical scavenging capacity. For example, tamarind leaf (*Tamarindus indica*) fractions with the highest phenolic content also exhibited the strongest DPPH antioxidant activity (Nainggolan et al., 2024). This relationship supports the assumption that phenolics are the main contributors to radical scavenging activity. Besides classical phenolics, eugenol, the principal aromatic compound in clove, has also been reported to possess strong antioxidant activity against DPPH and other radicals (Shende & Shilpashree, 2024). This study found that extracts rich in eugenol showed high radical inhibition percentages, indicating that aromatic components contribute significantly to antioxidant activity (Watuguly et al., 2023).

While polar solvents such as methanol or ethanol are generally more effective at extracting higher total phenolics and often yield lower IC_{50} values in DPPH assays, the use of semi-polar solvents like chloroform offers advantages. Semi-polar fractions extract compounds with intermediate polarity that also possess antioxidant activity, thus remaining a viable source of natural antioxidants (Shende & Shilpashree, 2024). These results reinforce the notion that clove leaves, often underutilized in the distillation industry, contain significant bioactive potential. From a sustainability and green industry perspective, utilizing clove leaves as a source of antioxidant compounds adds economic value and supports applications in pharmaceuticals, functional foods, and cosmetics. To strengthen the findings and clarify the active components responsible for antioxidant activity, further studies employing molecular identification techniques such as gas chromatography–mass spectrometry (GC-MS) or liquid chromatography–mass spectrometry (LC-MS) are recommended (Wael, 2023). Additionally, complementary antioxidant assays such as ABTS or FRAP are advised to provide a more comprehensive assessment of the extract's antioxidant capacity, as each method varies in sensitivity to different compound classes (Nainggolan et al., 2024).

CONCLUSION

The chloroform extract of clove leaf waste (*Syzygium aromaticum*) contains bioactive compounds including phenolics, flavonoids, tannins, and terpenoids with potential as natural antioxidants. The antioxidant activity of the extract was demonstrated by its ability to neutralize free radicals, yielding an IC_{50} value of 69.40 ppm in the DPPH assay, categorized as strong antioxidant activity. These results indicate that clove leaf waste, which is often underutilized, can serve as a valuable source of bioactive compounds. Therefore, the chloroform extract of clove leaf waste has potential for development as a raw material in pharmaceutical, functional food, and cosmetic products based on natural ingredients, while also supporting sustainability and agricultural waste management. Further research on active compound identification and additional antioxidant activity assays is strongly recommended to substantiate these findings.

AUTHORS CONTRIBUTION

Wael, S., Arini I., Muskita, M & Rakuasa, H. Designed and conducted the study, analyzed and interpreted the data, and wrote a draft of the manuscript. Interpreted the data and reviewed the draft manuscript, and supervised the entire process.

CONFLICT OF INTEREST

The authors declare no conflicts of interest, and will take full responsibility for the content of the article, including implications of AI-generated art.

REFERENCES

Blois, M. S. (1958). Antioxidant Determinations by The Use of a Stable Free Radical. *Nature, 181*, 1199-1200. https://doi.org/10.1038/1811199a0

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a Free Radical Method to Evaluate Antioxidant Activity. *LWT-Food Science And Technology, 28*(1), 25-30. https://doi.org/10.1016/s0023-6438(95)80008-5

Gulcin, I. (2011). Antioxidant Activity of Eugenol: a Structure-Activity Relationship Study. *Journal of Medicinal Food, 14*(9), 975-985. https://doi.org/10.1089/jmf.2010.0197

Hagerman, A. E., Riedl, K. M., Jones, G. A., Sovik, K. N., Ritchard, N. T., Hartzfeld, P. W., & Riechel, T. L. (1998). High Molecular Weight Plant Polyphenolics (Tannins) As Biological Antioxidants. *Journal Of Agricultural And Food Chemistry, 46*(5), 1887-1892. https://doi.org/10.1021/jf970975b

Mamahit, S. M. N., Katja, D. G., & Suryanto, E. (2025). Antioxidant activity and toxic properties test of distilled clove leaf (*Syzygium aromaticum)* waste. *Chemistry Progress, 18*(1). https://doi.org/10.35799/cp.18.1.2025.62657

Muderawan, I. W., Laksmi, P. P. D. S., Mudianta, I. W., & Martiningsih, N. W. (2025). Chemical Constituent And Antioxidant Activity Of Clove (Syzygium Aromaticum) Bud And Leaf Essential Oils From Bali. *Indonesian Journal Of Chemical Research*. https://doi.org/10.30598/ijcr.2025.12-mud

Molyneux, P. (2004). The Use Of The Stable Free Radical Diphenylpicrylhydrazyl (DPPH) For Estimating Antioxidant Activity. *Songklanakarin Journal of Science And Technology, 26*(2), 211-219. https://doi.org/10.1039/c3ay40367j

Nainggolan, R. M., Rahayu, M. P., & Rejeki, E. S. (2024). Antioxidant activity test, total flavonoid content, and total phenolic content of extract and fractions of tamarind leaves. *Jurnal Mandala Pharmacon Indonesia, 10*(2), Article 554. https://doi.org/10.35311/jmpi.v10i2.554.

Nusaly, W. N., Watuguly, T. W., Pattiasina, E. B., & Wael, S. (2023). Identification Of Medicinal Plants And Benefits In The Village Of Negeri Lima, Maluku District. *Biopendix: Jurnal Biologi, Pendidikan Dan Terapan. Universitas Pattimura*. *10*(1), 156-170. https://doi.org/10.30598/biopendixvol10issue1page156-170

Padayatty, S. J., Katz, A., Wang, Y., Eck, P., Kwon, O., Lee, J. H., Levine, M. (2003). Vitamin C As An Antioxidant: Evaluation Of Its Role In Disease Prevention. *Journal of The American College Of Nutrition, 22*(1), 18-35. https://doi.org/10.1080/07315724.2003.10719272

Pietta, P.G. (2000). Flavonoids As Antioxidants. *Journal Of Natural Products, 63*(7), 1035-1042. https://doi.org/10.1021/np9904509

Prior, R. L., Wu, X., & Schaich, K. (2005). *Standardized Methods For The Determination Of Antioxidant Capacity And Phenolics In Foods And Dietary Supplements*. Journal of Agricultural And Food Chemistry, 53(10), 4290-4302. https://doi.org/10.1021/jf0502698

Shahidi, F., & Ambigaipalan, P. (2015). Phenolics And Polyphenolics In Foods, Beverages And Spices: Antioxidant Activity And Health Effects-A Review. *Journal of Functional Foods, 18*, 820-897. https://doi.org/10.1016/j.jff.2015.06.018

Shahidi, F., & Ambigaipalan, P. (2015). Phenolics And Polyphenolics In Foods, Beverages And Spices: Antioxidant Activity And Health Effects-A Review. *Journal of Functional Foods, 18*, 820-897. https://doi.org/10.1016/j.jff.2015.06.018

Shende, S., & Shilpashree, B. G. (2024). *Extraction of Eugenol In Clove Extract And Evaluation Of Its Antioxidant Activity*. International Journal Of Engineering Applied Sciences And Technology, 9(2), 262–267. Https://Doi.Org/10.22161/Ijeab.92.29.

Wael, S., Nuringtyas, T. R., Wijayanti, N., & Astuti, P. (2018). Secondary Metabolites Production In Clove (Syzygium Aromaticum): Chemical Compounds. Biological Journal. https://doi.org/10.3923/jbs.2018.399.406

Wael, S., Watuguly, T. W., Arini, I., Smit, A., Matdoan, N., Prihati, D. R., & Astuti, P. (2018). Potential Of Syzygium Aromaticum (Clove) Leaf Extract On Immune Proliferation Response In Balb/C Mice

Infected With Salmonella Typhimurium.*Case Reports In Clinical Medicine*,*7*(12), 613-627. https://doi.org/10.4236/crcm.2018.712057

Wansi, S., & Wael, S. (2014). Analysis of chlorine content in tea bags based on brewing time. *Biopendix: Journal of Biology, Education and Applied Science, 1*(1), 22-31. https://doi.org/10.30598/biopendixvol1issue1page22-31

Watuguly, T. W., Leiwakabessy, F., Rehena, J. F., Mose, W., & Wael, S. (2022). Prevention Of Breast Cancer With The Breast Self-Examination Method (BSE) In The Negeri Lima Village, Leihitu District, Maluku Province.*Bioedupat: Pattimura Journal Of Biology And Learning*, *2*(2), 32-36. https://doi.org/10.30598/bioedupat.v2.i2.pp32-36

Wael, S. (2019). Potential of n-hexane extract of clove leaves (*Syzygium aromaticum* (L.)) on the sexual behavior of male rats (*Rattus norvegicus* Berkenhout, 1769) (Doctoral dissertation, Universitas Gadjah Mada).

Wael, S., Leiwakabessy, F., Mose, W., & Watuguly, T. W. (2023). Potential of Clove Extract (Syzygium Aromaticum) On Spermatozoa Rats (Sprague Dawley) Exposed to Arak Ambon (Sopi). *Biopendix: Jurnal Biologi, Pendidikan Dan Terapan*, *9*(2), 211-221. https://doi.org/10.30598/biopendixvol9issue2page211-221.