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Effect of Extraction Time and Sodium Bisulfite on Citronella (*Cymbopogon nardus*) Extract: Yields, Moisture, and GC-MS Analysis

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Abstract. This study investigated the influence of extraction time and sodium bisulfite concentration on citronella (*Cymbopogon nardus*) extracts using ethanol as solvent. Nine treatments (P1–P9) were evaluated for yield, moisture content, and preliminary TLC screening. The highest yield (59.94%) was obtained at 4 h with 2.5 g sodium bisulfite, while P8 (5 h, 2 g sodium bisulfite) exhibited a lower yield but higher moisture content, suggesting greater solubilization of polar compounds. ANOVA confirmed that extraction parameters significantly affected extract composition ($p < 0.05$). TLC results, however, produced anomalous Rf values exceeding the theoretical limit, attributed to the use of n-hexane: acetic acid (6:4) as mobile phase, which was unsuitable for terpenoid separation. Consequently, TLC was considered qualitative only. GC–MS analysis of P8 revealed 18 peaks dominated by long chain fatty acids (palmitic, oleic, cis vaccenic acids), while citronella's characteristic terpenoids (citronellal, citronellol, geraniol) were absent, confirming volatilization losses during ethanol extraction. These findings highlight that ethanol extraction at elevated temperatures yields fatty acid rich fractions with potential functional applications, however, it is unsuitable for producing authentic citronella essential oil. Future work should optimize TLC solvent systems, extend GC–MS profiling to all treatments, and employ alternative extraction methods such as steam distillation or solvent free microwave techniques to preserve volatile terpenoids.

Keywords: *Citronella; Ethanol Extraction; GC-MS*

Abstrak. Penelitian ini mengkaji pengaruh lama waktu ekstraksi dan konsentrasi natrium bisulfit terhadap ekstrak sereh wangi (*Cymbopogon nardus*) dengan menggunakan etanol sebagai pelarut. Sembilan perlakuan (P1–P9) dievaluasi berdasarkan rendemen, kadar air, serta uji pendahuluan KLT. Rendemen tertinggi (59,94%) diperoleh pada ekstraksi 4 jam dengan penambahan 2,5 g natrium bisulfit, sedangkan perlakuan P8 (5 jam, 2 g natrium bisulfit) menunjukkan rendemen lebih rendah namun kadar air lebih tinggi, yang mengindikasikan peningkatan kelarutan senyawa polar. Analisis ANOVA mengonfirmasi bahwa parameter ekstraksi berpengaruh nyata terhadap komposisi ekstrak ($p < 0,05$). Namun, hasil KLT menghasilkan nilai Rf yang tidak sesuai (melebihi batas teoritis), yang disebabkan oleh penggunaan fase gerak n-heksana: asam asetat (6:4) yang kurang tepat untuk pemisahan terpenoid. Oleh karena itu, KLT hanya dipertimbangkan sebagai analisis kualitatif. Analisis GC–MS pada perlakuan P8 menunjukkan 18 puncak yang didominasi oleh asam lemak rantai panjang (asam palmitat, oleat, cis vaksenat), sementara terpenoid khas sereh wangi (sitronelal, sitronelol, geraniol) tidak terdeteksi, menegaskan adanya kehilangan senyawa volatil selama ekstraksi etanol. Temuan ini menyoroti bahwa ekstraksi etanol pada suhu tinggi menghasilkan fraksi kaya asam lemak dengan potensi aplikasi fungsional, tetapi tidak sesuai untuk menghasilkan minyak atsiri sereh wangi yang autentik. Penelitian selanjutnya disarankan untuk mengoptimalkan sistem pelarut KLT, memperluas profil GC–MS pada seluruh perlakuan, serta menggunakan metode ekstraksi alternatif seperti destilasi uap atau teknik so*lvent free* microwave untuk mempertahankan terpenoid volatil.

Kata kunci: *Sereh wangi; Ekstraksi etanol; GC-MS*

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INTRODUCTION

Essential oils are complex mixtures of volatile aromatic compounds obtained from plants, typically through steam distillation or other selective extraction methods. They are widely applied in cosmetics, perfumery, aromatherapy, pharmaceuticals, and natural pesticides due to their distinctive fragrance and bioactivity (Ekpenyong & Akpan, 2017; Oliveira et al., 2015). Indonesia is one of the world's largest producers of essential oils, with citronella (*Cymbopogon nardus* and *Cymbopogon winterianus*) occupying a prominent position because of its economic value and characteristic terpenoid constituents such as citronellal, citronellol, and geraniol (Lawrence, 2000; Puspawati et al., 2016).

Despite its importance, the extraction of citronella oil remains challenging. Conventional steam distillation is effective for recovering volatile terpenoids but requires high energy input and may cause thermal degradation. Ethanol-based extraction has been proposed as an alternative, yet its selectivity toward volatile compounds is limited, often yielding fatty acid-rich fractions rather than pure essential oils (Ramadan, 2015; Barros & Silva, 2016).

Previous studies have examined the influence of extraction conditions on yield and composition (Kurniawan et al., 2020; Sugita et al., 2022), but systematic evaluation of extraction time combined with sodium bisulfite addition using ethanol solvent remains scarce. Previous comparative studies have also shown that solvent choice and extraction duration strongly influence phytochemical recovery (Do et al., 2014; Ariyani et al., 2017), while GC–MS based identification of essential oil components has been standardized in essential oil research to ensure accurate chemical profiling (Adams, 2007; Jirovetz et al., 2006). Several reports further highlight antimicrobial and antifungal activities of citronella oil and related species, reinforcing its functional importance beyond fragrance applications (Bassolé & Juliani, 2012; Wannissorn et al., 2005). The role of harvest stage and distillation parameters has also been emphasized in lemongrass oil studies, showing that process conditions critically affect essential oil yield and quality (Rajeswara Rao et al., 2005; Satyal et al., 2012).

According to research by Kurniawan et al. (2020), the longer the extraction time, the less the product yield increases, and the addition of sodium bisulfite aims to obtain the maximum citronellal content. Their study with extraction time variables of 3, 3.5, 4, 4.5, and 5 hours and sodium bisulfite variations of 10, 15, 20, 25, and 30 grams showed that the best quality lemongrass oil was obtained at an extraction time of 4 hours with the addition of 20 grams of sodium bisulfite. Similarly, Muyassaroh (2012) reported that the amount of sodium bisulfite added greatly affects the citronellal content because lemongrass oil is not soluble in water, including the citronellal it contains. Her research results demonstrated that the best quality of lemongrass oil was achieved with the addition of 20 grams of sodium bisulfite and a stirring speed of 150 rpm, yielding a citronella content of 40.35%, a geraniol content of 40.26%, and a density of 0.8867 g/mL.

This study aims to address that gap by investigating how variations in ethanol extraction time and sodium bisulfite concentration affect the yield and phytochemical profile of citronella extract. By employing Thin Layer Chromatography (TLC) and Gas Chromatography-Mass Spectrometry

(GC-MS), the research seeks to clarify whether ethanol extraction can preserve citronella's key terpenoid markers or whether process modification is required to optimize recovery of its characteristic volatile constituents.

MATERIAL AND METHODS

Materials

Fresh citronella (*Cymbopogon nardus*) leaves and stems were collected from Bandung Barat, Indonesia. Ethanol 70% was used as the extraction solvent, and sodium bisulfite ($NaHSO_3$) was added to enhance citronellal recovery. All chemicals were of analytical grade.

Equipment

Extraction was performed using a three-necked round bottom flask equipped with a magnetic stirrer and a hot plate. Distillation was carried out with a standard distillation apparatus. TLC analysis employed silica gel F254 plates, while GC–MS analysis was conducted using a capillary column suitable for essential oil profiling.

Experimental Design

The study investigated two independent variables: extraction time (3, 4, and 5 h) and sodium bisulfite concentration (1.5, 2.0, and 2.5 g). Nine treatment combinations were tested (A–I), each replicated twice, resulting in 18 experimental units. A Completely Randomized Design (CRD) was applied, and data were analyzed using ANOVA to determine the significance of treatment effects (Kurniawan et al., 2020; Rahmadani et al., 2018).

Procedure

Samples (100 g dried citronella) were soaked in 400 mL ethanol 70% for 72 h, then subjected to extraction at 80 °C for 3–5 h under continuous stirring (150 rpm). The filtrate was distilled at 80 °C for 2 h to separate ethanol and concentrate the extract. Sodium bisulfite was added post distillation (1.5–2.5 g), stirred for 2 min, and allowed to settle into two phases. Optimization of extraction conditions has been compared across rosemary and other aromatic plants (Bousbia et al., 2009).

TLC was performed using silica gel F254 plates with n-hexane: acetic acid (6:4) as the mobile phase. Samples were visualized under UV light and iodine vapor. Rf values were calculated relative to solvent front migration. However, anomalous Rf values (>1.0) were observed, attributed to the unsuitable polarity of the chosen solvent system for terpenoid separation. Thus, TLC was considered qualitative screening only, consistent with previous reports emphasizing the importance of solvent selection in phytochemical separation (Sugita et al., 2022).

GC–MS analysis was conducted on the extract from treatment H (P8: 5 h, 2 g $NaHSO_3$), representing the longest extraction condition. The GC was programmed with a temperature gradient optimized for volatile and semi-volatile compounds. Mass spectra were compared against reference libraries for compound identification. Reference methods for TLC and GC–MS separation of terpenoids have been widely reported (Tzakou et al., 2006; Bayala et al., 2014). The analysis revealed fatty acid dominance, confirming volatilization losses of citronella terpenoids during ethanol extraction (Ramadan, 2015; Barros & Silva, 2016).

RESULT AND DISCUSSION

Extraction Yield and Moisture Content

The extraction yield varied across treatments, with the highest yield (59.94%)

obtained in P6 (4 h, 2.5 g $NaHSO_3$). However, yield alone does not determine extract quality. Treatment P8 (5 h, 2 g $NaHSO_3$) produced a lower yield (26.37%) but exhibited relatively high moisture content (14.58%), as shown in Figure 1. Elevated moisture suggests greater solubilization of polar and semi-polar compounds, which may enrich the chemical complexity of the extract. Previous studies have emphasized that prolonged extraction can increase solvent penetration and compound recovery, but excessive duration may also lead to volatilization losses and degradation of thermolabile terpenoids (Kurniawan et al., 2020; Rahmadani et al., 2018). Thus, P8 was considered representative of an "extreme extraction condition" suitable for further chemical profiling.

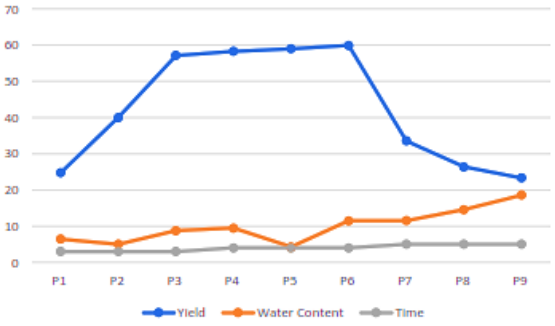

Figure 1. Effect of time on yield and moisture content

Thin Layer Chromatography (TLC) Analysis

TLC was performed to provide preliminary screening of citronella extracts under different extraction times and sodium bisulfite concentrations. The Rf values obtained varied across treatments, with P8 (5 h, 2 g $NaHSO_3$) showing the highest reported value (4.5), as shown in Table 1. However, this exceeds the theoretical limit of 1.0, indicating methodological inconsistency. The anomaly can be attributed to the choice of mobile phase, n-hexane: acetic acid (6:4), which is overly polar for citronella terpenoids. This solvent system promotes excessive migration of semi-polar compounds, leading to invalid Rf values.

Table 1. Rf value

Treatment	Extraction Time (hours)	$NaHSO_3$ (g)	Rf Value
P1	3	1.5	0.9
P2	3	2	1
P3	3	2.5	1.1
P4	4	1.5	1.8
P5	4	2	1.6
P6	4	2.5	1.9
P7	5	1.5	3.2
P8	5	2	4.5
P9	5	2.5	2

Previous studies have highlighted that solvent systems such as n-hexane: ethyl acetate or chloroform: methanol are more appropriate for separating terpenoid constituents (Rahmadani et al., 2018; Sugita et al., 2022). Therefore, while TLC provided visual evidence of separation, the numerical Rf values cannot be considered reliable indicators of extract quality. For this reason, TLC was treated only as a qualitative screening tool, and GC–MS analysis was prioritized for definitive chemical profiling.

The selection of P8 for GC–MS was not based solely on TLC values but rather on its extraction condition (longest duration with sodium bisulfite addition) and statistical significance confirmed by ANOVA (Table 2). This approach ensured that the sample represented an "extreme extraction condition," allowing evaluation of whether prolonged ethanol extraction could preserve citronella's volatile terpenoids. The subsequent GC–MS results confirmed that despite apparently optimal TLC separation, the extract was dominated by long chain fatty acids rather than citronella markers, underscoring the

methodological limitations of ethanol extraction for volatile compounds.

ANOVA analysis confirmed that extraction time and sodium bisulfite concentration significantly influenced citronella extract composition (F value 7.95 > F table 3.22, $p < 0.05$). This statistical evidence supports the rationale for selecting P8 as a sample for GC–MS analysis. By choosing the longest extraction time with sodium bisulfite addition, the study aimed to evaluate whether statistically significant conditions also corresponded to optimal phytochemical recovery. Similar approaches have been reported in essential oil research, where extreme treatments are analyzed to reveal methodological limitations (Sugita et al., 2022), and also the design of distillation apparatus and industrial scale extraction has been discussed in Indonesian contexts (Wahyudi et al., 2018; Putri et al., 2021).

Table 2. ANOVA summary

Source	df	SS	MS	F Hit	F Table 5%	Result
Treatment	8	22.47	2.81	7.95	3.22	Significant
Error	9	3.18	0.35	—	—	—
Total	17	25.65	—	—	—	—

GC-MS Analysis

GC–MS of P8 revealed 18 peaks (Figure 2), dominated by long chain fatty acids such as palmitic acid, oleic acid, and cis vaccenic acid. Characteristic citronella terpenoids (citronellal, citronellol, geraniol) were not prominent, indicating volatilization losses during ethanol extraction.

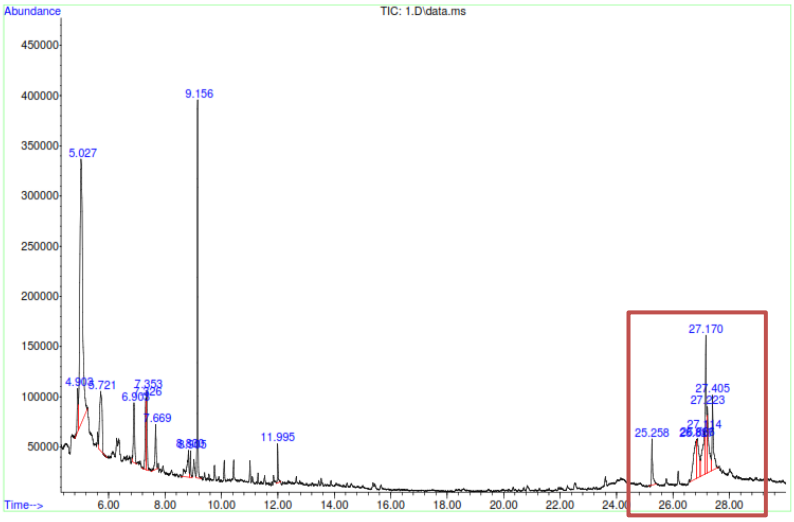

Figure 2. GC-MS results

The GC-MS results above show 18 compound peaks found in the eight (8) treatment samples. Of all the compound peaks, no compounds characteristic of lemongrass were found. This occurred because, during the extraction process, evaporation of the solvent took place, due to the fact that citronella contains two compounds, namely citronellal and citronellol, which are terpenoid compounds with a distinctive citrus aroma. Terpenoid compounds generally have volatile properties, where they tend to evaporate at room temperature or relatively low temperatures. Due to its volatility, citronella can be lost during storage or processing if not handled properly. In an ethanol-lemongrass mixture, citronella becomes more volatile, which can affect the

final concentration of the compound in the product. Although citronella was not found in the essential oil content tested, other compounds were detected in the GC-MS test. There were 18 peaks, each of which had 3 compounds detected in the GC-MS test. Not all of the peaks obtained played a role in essential oils; there were 7 peaks (boxed) that contained long-chain fatty acids. Fatty acid profiling in essential oils has been linked to antioxidants and antimicrobial properties (Barros et al., 2017; Kumar et al., 2012). Palmitic and oleic acid esters are known to enhance stability and emollient properties in cosmetic formulations (Penfold et al., 2014; Rizal, 2010). The compounds with fatty acid content detected in the GC-MS analysis results are shown in the following table 3.

Table 3. Main GC-MS peaks

Peak	Compound	Properties
12	Hexadecanoic acid	Emollient, antioxidant
13	2,3-dihydroxypropyl palmitate	Stabilizer
14	Cis-vaccenic acid	Anti-inflammatory
15	Oleic acid	Emollient
16	Oleic acid isomer	Emollient
17	Long-chain fatty acid	Antimicrobial

Fatty acids were detected at peaks 12, 13, 14, 15, 16, and 17. At peak 12, the detected compound was Hexadecanoic acid (CAS). Kumar et al. (2015) mentioned that lemongrass oil has potential benefits in improving the oxidative stability, texture quality, and emollient properties of the oil. Hexadecanoic acid, 2,3-dihydroxypropyl ester (known as palmitic acid, 2,3-dihydroxypropyl ester) is an ester form of palmitic acid and glycerol. Penfold et al. (2014) state that palmitic acid and glycerol esters Are often used to improve product stability, provide emollient effects, and improve product texture. In lemongrass oil, esters can help improve the quality and effectiveness of lemongrass oil-based cosmetic formulations. Cis-Vaccenic acid is a long-chain unsaturated fatty acid with a double bond at the 11th position from its methyl end. , is a form of omega-7 fatty acid found in various vegetable oils and animal fats Cis vaccenic acid and related long chain fatty acids contribute to anti inflammatory activity in essential oils (Hsu et al., 2016; Oliveira et al., 2015). Hsu et al. (2016) stated that cis-vaccenic acid can improve the quality of skin care products and essential oils by improving skin moisture and providing anti-inflammatory and antioxidant effects. Oleic Acid appears in two identifications, namely Hit 15 and Hit 16. Wijaya (2018) mentions that oleic acid is a monounsaturated fatty acid commonly found in olive oil and various other vegetable oils.

Ethanol is known to preferentially extract semi-polar lipids rather than volatile monoterpenes, which explains the absence of citronella markers (Ramadan, 2015; Barros & Silva, 2016). While fatty acids contribute to antioxidant, emollient, and antimicrobial properties (Dangol et al., 2023), their dominance here confirms that the product is better described as an ethanol extract rather than a true essential oil.

Critical Interpretation

The combined data demonstrate that yield maximization (P6) did not correspond to phytochemical preservation, while P8, despite lower yield, provided a chemically complex extract that revealed the limitations of ethanol extraction. The statistical significance of treatment effects, coupled with GC–MS confirmation, underscores that ethanol extraction at elevated temperatures is unsuitable for preserving citronella's volatile terpenoids. Alternative methods such as steam

distillation, solvent free microwave extraction, or vacuum assisted techniques are recommended to retain citronellal and related compounds (Suryanti et al., 2024). Thus, P8 serves as a critical sample illustrating that extraction conditions deemed “optimal” by yield or TLC do not guarantee preservation of citronella’s characteristic volatile profile.

CONCLUSION

This study demonstrated that extraction time and sodium bisulfite concentration significantly influenced citronella extract yield and moisture content, with ANOVA confirming the statistical relevance of treatment variations. Although TLC was employed as a preliminary screening tool, the use of n-hexane: acetic acid (6:4) as the mobile phase produced anomalous Rf values exceeding the theoretical limit, underscoring the methodological limitations of this solvent system for terpenoid separation. Consequently, TLC results were considered qualitative rather than quantitative.

The GC–MS analysis of treatment P8 (5 h, 2 g $NaHSO_3$), selected as a representative extreme extraction condition, revealed that the extract was dominated by long-chain fatty acids rather than citronella’s characteristic volatile terpenoids. This finding highlights the inability of ethanol extraction at elevated temperatures to preserve citronellal, citronellol, and geraniol, despite apparently optimal extraction conditions.

Overall, the methodological limitations of ethanol extraction reaffirm earlier findings on solvent selectivity in essential oil recovery (Bruice, 2010; Lowry & Richardson, 1987; Dalimarta, 2000). The study confirms that while ethanol extraction can yield fatty acid rich fractions with potential antioxidant and cosmetic applications, it is unsuitable for producing authentic citronella essential oil. Future research should employ alternative extraction methods such as steam distillation, solvent free microwave extraction, or vacuum assisted techniques to optimize the recovery of volatile terpenoid markers.

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