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Breaking Boundaries: A Comparative Analysis of Weak vs. Strong Bases in Eugenol Isolation from *Syzygium aromaticum* (L.) Merr. & LM Perry

Dwi Handayani[1], Rina SE Sitindaon[2], Nurul Hidayati[3], Arif Setiawansyah[3,4*]

[1]Faculty of Pharmacy, Universitas Kader Bangsa, Palembang, Indonesia
[2]Faculty of Health, Universitas Kader Bangsa, Palembang, Indonesia
[3]Akademi Farmasi Cendikia Farma Husada, Bandar Lampung, Indonesia
[4]Laboratorium Sentra Ekstrak, Akademi Farmasi Cendikia Farma Husada, Bandar Lampung, Indonesia

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*Corresponding author e-mail: arif12.setiawansyah@gmail.com

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ABSTRACT

***Introduction**: Eugenol, a phenolic compound classified within the phenylpropanoid group, stands out as a key constituent of clove oil, boasting a myriad of biological activities. Its isolation typically involves the utilization of an alkaline solution, yet the choice of alkaline agent significantly impacts the yield of eugenol. This study delves into the impact of various alkaline solutions on the isolated eugenol content from Syzygium aromaticum essential oil, leveraging its acidic properties to form a soluble eugenol salt in an aqueous medium. **Methods**: Employing alkaline solutions with a concentration of 1 N, including both strong bases (such as KOH, NaOH, and $Ba(OH)_2$) and weak bases (like $Ni(OH)_2$, $Al(OH)_3$, and $Zn(OH)_2$), we scrutinized the diverse outcomes on eugenol content. **Result**: Results revealed that KOH yielded the highest eugenol content at 96.91%, while $Zn(OH)_2$ displayed the lowest at 20.99%. **Conclusion**: Nevertheless, the potential of weak alkaline solutions in the eugenol isolation process from Syzygium aromaticum essential oil remains noteworthy. Future endeavors should focus on optimizing the ideal concentration of weak alkaline solutions for this purpose.*

***KEYWORDS**: Eugenol, isolation, clove oil, alkaline, GC-MS*

INTRODUCTION

Eugenol (4-allyl-2-methoxyphenol) is a phenolic compound belonging to the phenylpropanoid group, which is one of the major components of clove oil (*Syzygium aromaticum* (L.) Merr. & LM Perry) (Zhang et al., 2013). Eugenol finds wide application in various industries such as pharmaceuticals, cosmetics, and food (Chatterjee & Bhattacharjee, 2015). Apart from being abundant in clove oil, eugenol is also found in various essential oils of plants, both vegetables and spices, such as basil (4.2-4.97 mg/g), laurel bay (1.34 mg/g), turmeric (2.1 mg/g), nutmeg (0.32 mg/g), and thyme (0.021 mg/g) (Raja et al., 2015).

Chemically, eugenol possesses a phenolic structure with a methoxy group and an allyl chain, which confers its numerous benefits. Various studies have reported its

pharmacological activities including antioxidant (Mittal et al., 2014), analgesic (Towaha, 2012), antimutagenic (Ivanovic & Harrison, 2015), antiplatelet (Kumar et al., 2012), anticancer (Huan et al., 2012), antiallergic (Mlcek et al., 2016), and anti-inflammatory (Gojani et al., 2023) properties. Additionally, eugenol has been reported to exhibit antimicrobial activity (Raja et al., 2015). Several studies indicate that eugenol can inhibit the growth of many human pathogenic microbes, including large groups of Gram-positive and Gram-negative bacteria (Al-Mariri & Safi, 2014), fungi (Didehdar et al., 2022), and a number of parasites, including *Giardia lamblia* (Hajare et al., 2022), *Fasciola gigantica* (Kumar et al., 2014), and *Haemonchus contortus* (Rinaldi et al., 2015). Eugenol can also protect against carbon tetrachloride-induced liver damage (Wibowo et al., 2017).

The broad pharmacological activities of eugenol have led to its extensive isolation for further research. Various eugenol isolation techniques have been developed, one of which involves the use of alkaline solutions (Khalil et al., 2017). The isolation of eugenol using alkaline typically relies on its weak acidic nature, allowing it to form water-soluble phenolate salts in alkaline conditions (Haryadi, 2017), facilitating the separation of eugenol from other components in essential oils (Putri et al., 2014). The use of bases in eugenol isolation has been widely adopted by many scientists, employing various types of bases such as NaOH, KOH, and $Ba(OH)_2$ (Putri et al., 2014; Khalil et al., 2017). Eugenol isolation with NaOH yielded a eugenol content of 82.6% (Lutfi et al., 2014), while with $Ba(OH)_2$ it was 71.33% (Putri et al., 2014). This indicates that the use of different bases results in different eugenol yields. However, to date, there have been no reports comparing the effectiveness of strong and weak bases in isolating eugenol from clove leaf oil.

Therefore, this study aims to compare the effectiveness of strong and weak bases in the process of isolating eugenol from clove leaf oil.

METHODS

Material

All chemicals and reagents used in this work were analytical grade. Clove leave essential oil (PT. Darjeeling Sembrani Aroma, Bandung; Batch No. DSA/BB-EO-029/LS-015), KOH (PT. ROFA Laboratorium Indonesia, Bandung), NaOH (PT. Smart Sumber Kimia Veteran, Palembang), $Ba(OH)_2$ (Ngalam Lab, Malang), $Ni(OH)_2$ (MaxLab, Tangerang), $Al(OH)_3$ (PT. ROFA Laboratorium Indonesia, Bandung), $Zn(OH)_2$ (Ngalam Lab, Malang), Aquades (PT. Smart Sumber Kimia Veteran, Palembang), H_2SO_4 (PT. Smart Lab Indonesia, Cilacap), *n*-heksana (PT. Smart Lab Indonesia, Cilacap).

Eugenol isolation

The isolation of eugenol was conducted using a method adapted from Putri et al. (2014)

with slight modifications. The isolation process began by reacting clove leaf oil with 1 N alkaline solutions of different types of bases including strong bases (KOH, NaOH, and $Ba(OH)_2$) and weak bases ($Al(OH)_3$, $Ni(OH)_2$, and $Zn(OH)_2$) at an oil-reactant ratio of 1:5. A volume of 50 mL of clove leaf oil was mixed with 250 mL of basic solution in an Erlenmeyer flask, then stirred with a magnetic stirrer for 30 minutes at 50°C. The mixture was then transferred to a separating funnel and left until two layers formed. The aqueous phase was then extracted and mixed with 1.5 N sulfuric acid. The mixture was stirred with a magnetic stirrer for 30 minutes at room temperature, then separated in a separating funnel. The organic phase was then taken for further analysis. The isolation process was conducted in three independent replications and the result was presented as mean ± SD followed by statistical analysis using One Way ANOVA, Tukey's test in a GraphPad Prism 10 version software.

Gas chromatography – mass spectrometry (GC-MS) analysis

The eugenol was analyzed using GC-MS, employing a Shimadzu QP2010 MS GC-MS equipped with an Rtx-5MS column (column length 30 mm; thickness 0.25 µm; diameter 0.25 mm). The operational conditions were as follows: column temperature 40°C, injection temperature 260°C, pressure 150 kPa, split ratio 1.0 with helium as the carrier gas.

RESULTS AND DISCUSSION

The isolation of eugenol from clove leaf oil was carried out chemically by exploiting the acid-base reaction of eugenol compound with an alkaline solution. Eugenol, being acidic in nature, undergoes a substitution reaction by replacing the released proton on the OH of phenolic group with the cation from the alkaline solution. The cation from the dissociation of the base in water readily attracts eugenol, forming eugenolate salt that is soluble in water (Figure 1). This facilitates the separation process of eugenol compound from other mono and sesquiterpene components present in clove leaf oil.

The formed eugenolate salt is then converted back into free eugenol by adding sulfuric acid (H_2SO_4) solution. When the eugenolate salt reacts with the H_2SO_4 solution, it undergoes hydrolysis, yielding eugenol cation and anion, which are subsequently substituted by H^+ from H_2SO_4 to form free eugenol (Figure 2). The resulting free eugenol can then be easily separated by liquid-liquid extraction using n-hexane. Our results demonstrate a clear distinction in eugenol yields between the strong base and the weak base extraction methods, with the highest yields observed with $Al(OH)_3$ and $Zn(OH)_2$. Conversely, the strong base yielded lower quantities of eugenol as shown in Figure 3. The weak base consistently produced higher eugenol yields. The yield discrepancy between strong and weak bases underscores the importance of considering the ionization

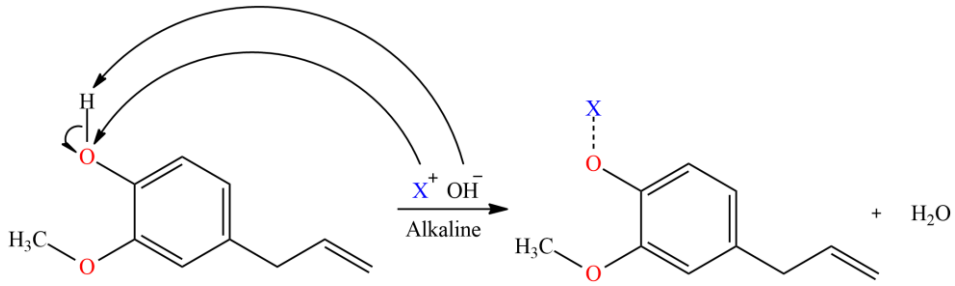

Figure 1. Mechanism of acid-base reaction between eugenol and alkaline solution forming eugenolate salt. Note: X represents alkaline ion (Nurdin, 2019).

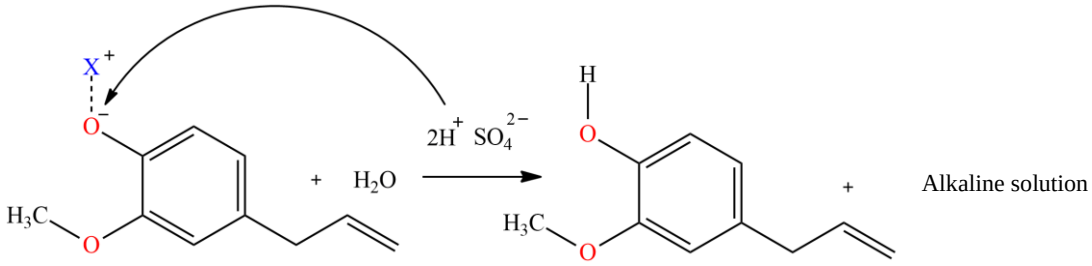

Figure 2. Neutralization reaction of eugenolate salt into a free eugenol (Nurdin, 2019).

potential when selecting a base for eugenol isolation. However, it is crucial to note that igher yields do not necessarily translate to superior quality. For instance, there is no correlation between yield and purity.

The purity of each isolate obtained was further analyzed by chromatography using GC-MS to observe the differences in effectiveness of each type of base used. The GC chromatogram (Figure 4) shows differences in peak intensity and the number of peaks observed, indicating variations in the concentration and number of compounds present in each analyzed sample. The certainty of the eugenol peak position was then confirmed from the MS data by comparing the MS spectra of each peak from the sample with the MS spectra from the WILEY7.LIB library. This provides information about the presence of eugenol peaks located at a retention time of 9.75 minutes. The validity of the eugenol peak appearing at a retention time of 9.75 minutes is marked by the similarity in fragmentation patterns between the compound at the observed peak in the sample and the data in the WILEY7.LIB database. As illustrated in Figure 4, the eugenol compound has a molecular ion at m/z 164 with a base peak ion at m/z 149. This fragmentation pattern has a similarity index of 97%, indicating that the peak is indeed the peak of the eugenol compound.

Overall, the GC-MS results revealed variations in the purity of eugenol in each isolate, with the highest yield obtained from KOH (96.91%) and the lowest from $Zn(OH)_2$ (20.99%), as shown in Figure 3b. These differences in results indicate that different types of bases also have varying effectiveness. Strong bases exhibit better effectiveness compared to weak bases due to the higher reactivity of strong bases compared to weak

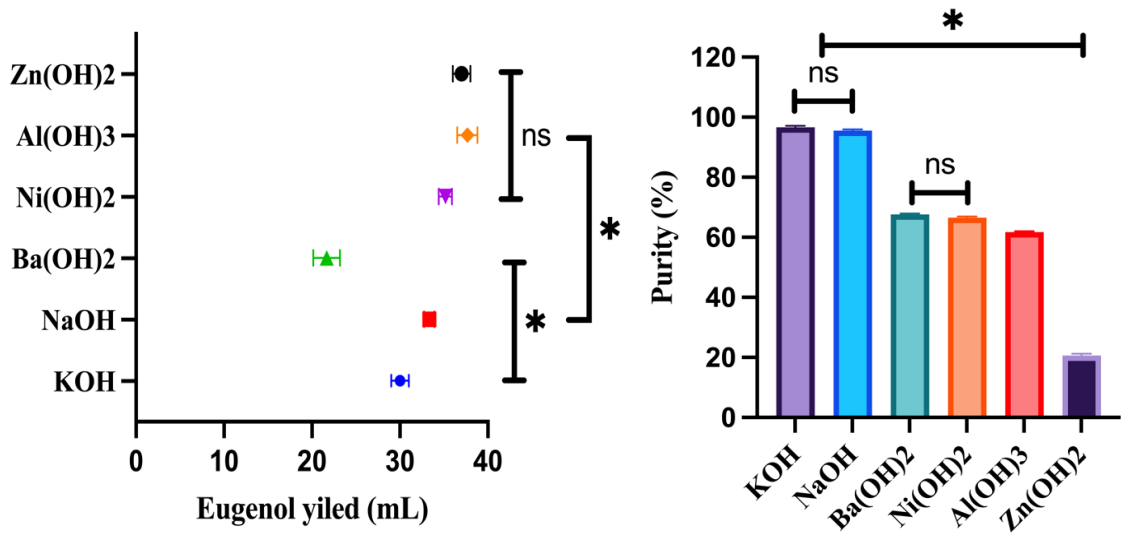

Figure 3. Result of eugenol isolation. (a) eugenol yield; (b) eugenol purity. *Data was significantly different at $p < 0.05$ (n=3).*

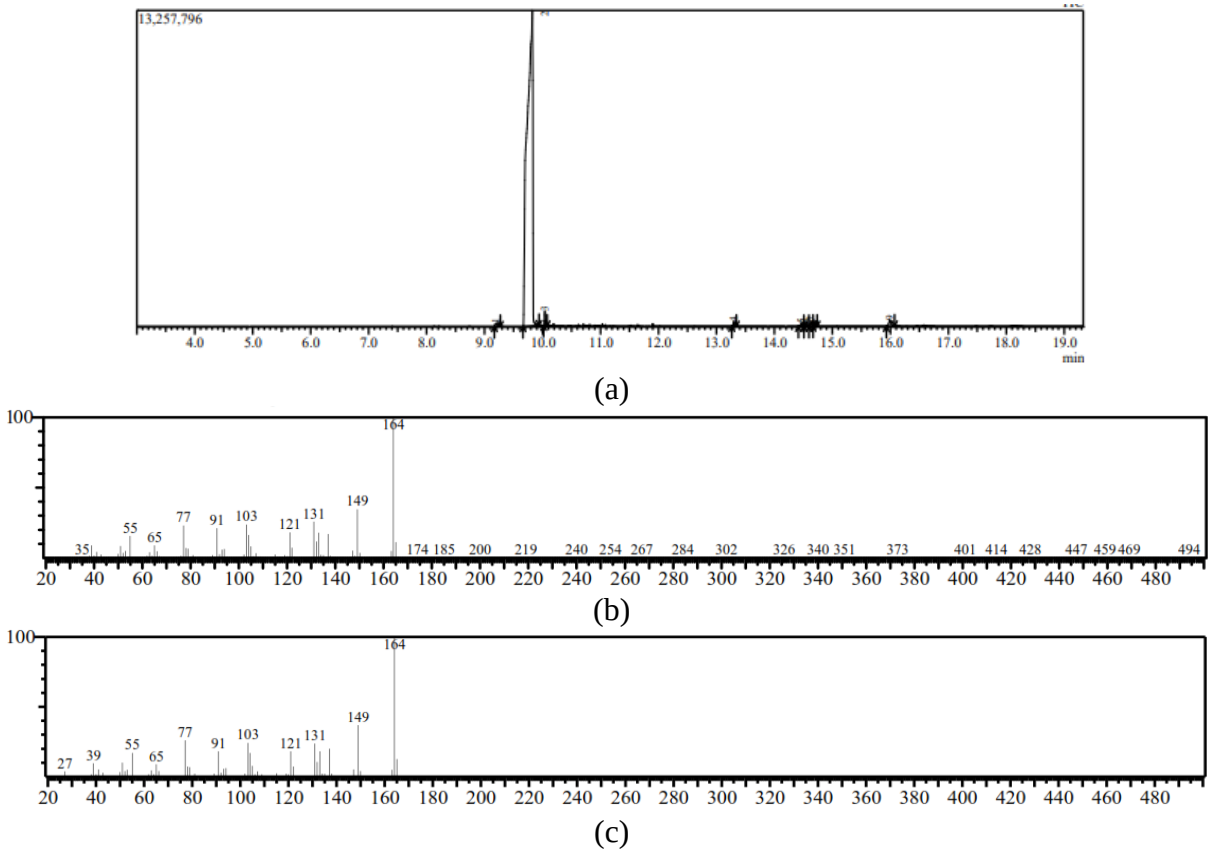

Figure 4. GC-MS result of isolate. (a) GC chromatogram (b) MS spectra of the compound peak at a retention time of 9.75 minutes and (c) MS spectra of the eugenol compound in the WILEY7.LIB database.

bases (Haas, 2023). The high reactivity of strong bases leads to an increased reaction rate between the cation of the base compound and eugenol, resulting in more effective formation of eugenolate salt. Additionally, differences are also observed in the alkali metals used, where eugenol with the highest purity is obtained from bases with alkali metal elements (KOH and NaOH). Meanwhile, eugenol obtained from bases with alkaline earth metal

elements ($Ba(OH)_2$) has relatively low purity, similar to the results from bases with elements from group IIIA ($Al(OH)_3$), and transition metals ($Ni(OH)_2$, and $Zn(OH)_2$). This is because alkali metals (group IA) have smaller atomic radii compared to alkaline earth metals (group IIA) and group IIIA, and transition metals, resulting in more effective ionization energy of alkali metals (Greenwood & Alan, 1997). Furthermore, elements in alkali metals only have one valence electron, whereas alkaline earth metals have two valence electrons. Thus, the ionization energy required to release one electron to form one ion is smaller than that required to release two electrons to form two ions (Lide, 2003). Nevertheless, weak bases or bases composed of alkaline earth or transition metals can still be used for isolating eugenol from clove leaf oil, albeit with lower purity. Therefore, optimization of the concentration and ratio between the oil and weak base is necessary for eugenol isolation to achieve better results.

CONCLUSION

The comparative study on the isolation of eugenol from *Syzygium aromaticum* using weak and strong bases has provided valuable insights into the efficiency and selectivity of different extraction methods. Our findings demonstrate that the choice of base significantly influences the yield and purity of the isolated eugenol. The weak base ($Al(OH)_3$, $Ni(OH)_2$, and $Zn(OH)_2$) exhibited higher extraction efficiency, resulting in greater eugenol yields compared to the strong base (KOH, NaOH and $Ba(OH)_2$). However, the strong base showed improved selectivity, leading to a purer eugenol extract with fewer impurities (purity up to 96.91%). This trade-off between yield and purity highlights the importance of considering the specific requirements of the intended application when selecting an isolation method. Future research should focus on exploring additional bases with intermediate strengths and investigating the potential of mixed-base systems to further optimize the isolation process. Moreover, scaling up these methods and assessing their economic viability will be crucial for industrial plications.

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