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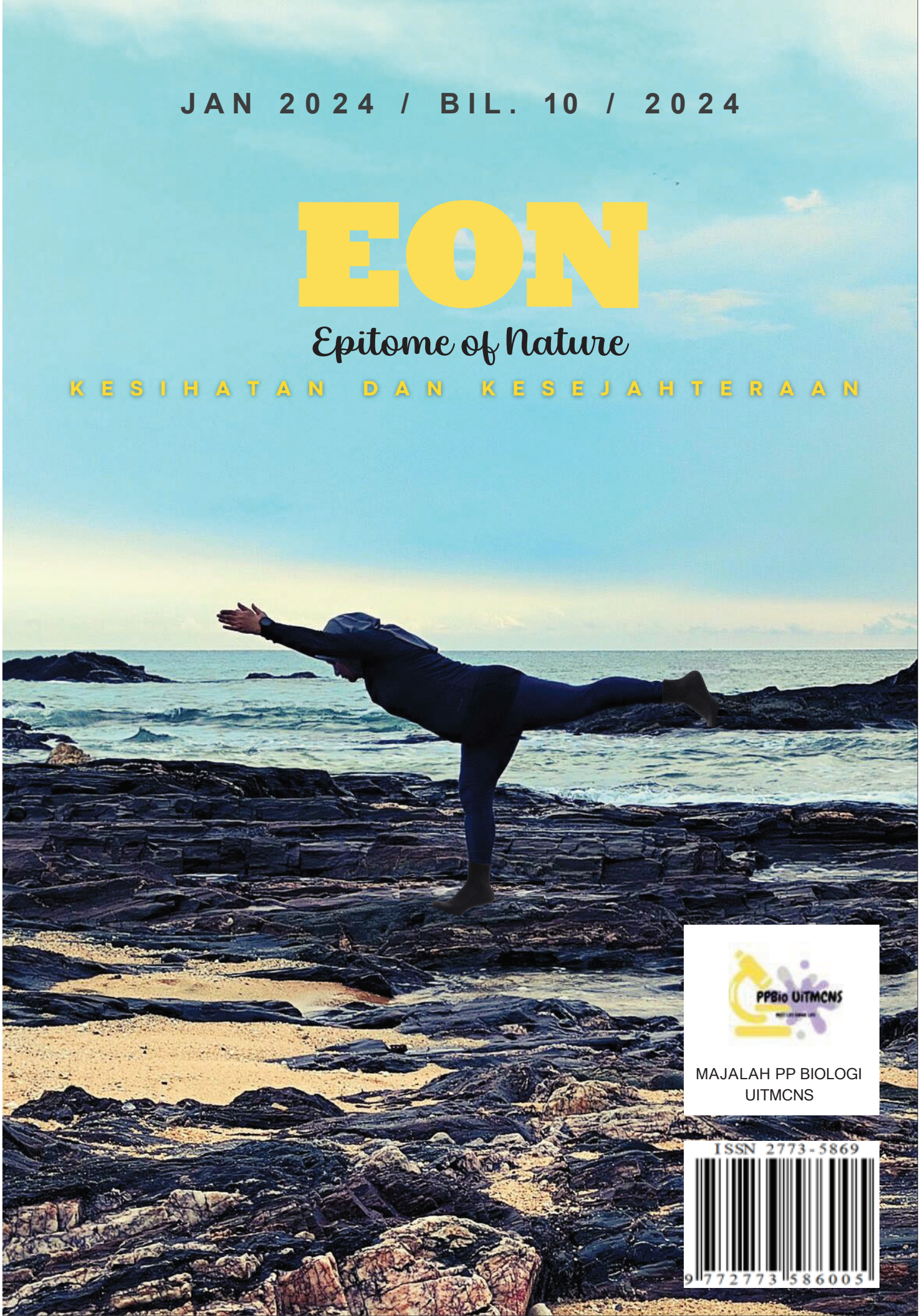

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THE ENCOUNTER WITH CLOVE FLOWER BUDS

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There are about 1200 species of plants in the *Syzygium* genus, belonging is well known as the clove orto the Myrtaceae family. *Syzygium aromaticum* cengkih, in Malay (Figure 1). The most important organ of *S. aromaticum* that is involved in folk medicines and traditional practices is the Faculty of Pharmacy, UiTM, the pharmacognosy lessons in the fifth week of the semester would include the introduction to clove oil as an example of a non-glyceride lipid. The brown clove flower buds are subjected to experimental and laboratory procedures, such as the introduction of the oil in plants.

Pattamadilok et al. (2021) established a quality specification of Thai clove samples by determining their physicochemical characteristics.

They would consist of the following percentages of its

- water content, not more than 7% (v/w)
- volatile oil content, not less than 23% (v/w)
- total ash content, not more than 7% (w/w)
- acid insoluble ash, not more than 1% (w/w)
- 95% ethanol extractive, not less than 19% (w/w), and
- water extractive, not less than 23% (w/w)

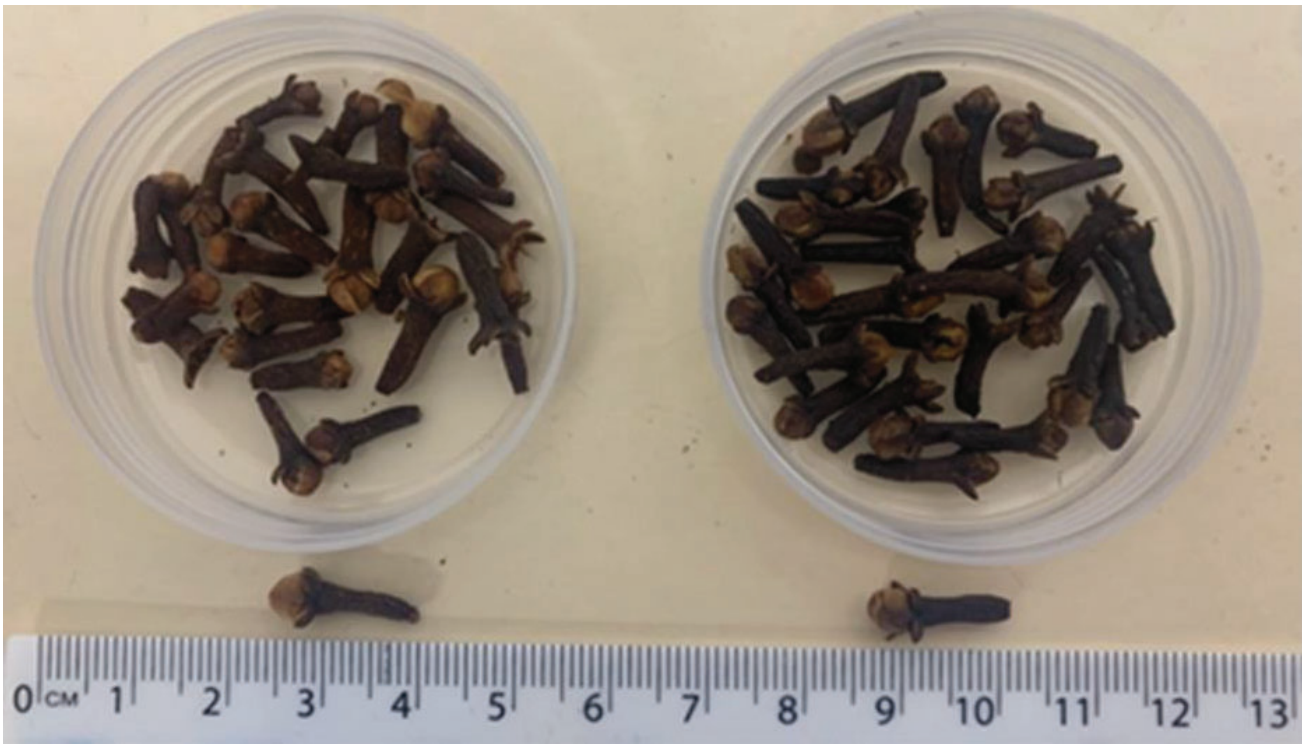


Figure 1: Two samples of dried clove flower bud from India (left) and unknown source (right), available in the local market (Source: authors' own collection)

The cloves could provide a naturally occurring oil, which is a mixture of volatile constituents, with a distinctive odour. It consists of mixtures of terpenes, which are unsaturated hydrocarbons and oxygenated derivatives, such as phenols, alcohols, ketones, aldehydes and esters. The clove oil is soluble in organic solvents and only partially soluble in water or aqueous alcohol. Therefore, it can be isolated from the clove specimen via extraction procedure, with non-polar solvents. The clove oil can also be obtained commercially, by steam distillation. In the laboratory practical, a distillation setup by using a Clevenger or Dean-Stark apparatus is arranged for the pharmacy undergraduates (Figure 2). It comprises of the round-bottom flask, a condenser and a Dean Stark trap. About half of a millilitre of xylene (dimethylbenzene, density = 0.87 g/ml) could be added via the graduated tube, to trap the clove oil. It is aimed the students could learn about the moisture content in the natural oil, from the crude botanical drug.

The Clevenger or Dean-Stark apparatus is a combination of a glass condenser and a flask, for continuous removal of the water, that is produced during reflux (Figure 2). It was invented for the determination of the water content in petroleum (Dean & Stark, 1920). It is a kind of indirect steam distillation instrumentation, whereby the steam is generated in situ (in the flask, Figure 2), and the water level could be kept below the clove material. The co-distillation technique uses live steam to separate components from the clove mixture. It is considered an effective method to extract the components from the clove essential oil with high boiling points, as high as 200°C.

Yet, the oil vapours are closer to 100°C, thus helping to preserve the compounds from degradation. This technique could allow the distillation to be performed at temperatures below the boiling points of the individual clove components (Figure 3).

In this case, the clove oil has a high boiling point, where the boiling point for eugenol is recorded as 254°C. Meanwhile, the vapor pressure of the oil at 100 °C, is much less. The clove oil forms an azeotrope with water and can be fractionally distilled at a lower temperature. Since the clove oil is immiscible with water, it can form a separate layer, which would allow an easy separation.

Some essential oils are heavier than water, such as clove essential oil (Asfaw, 2022).

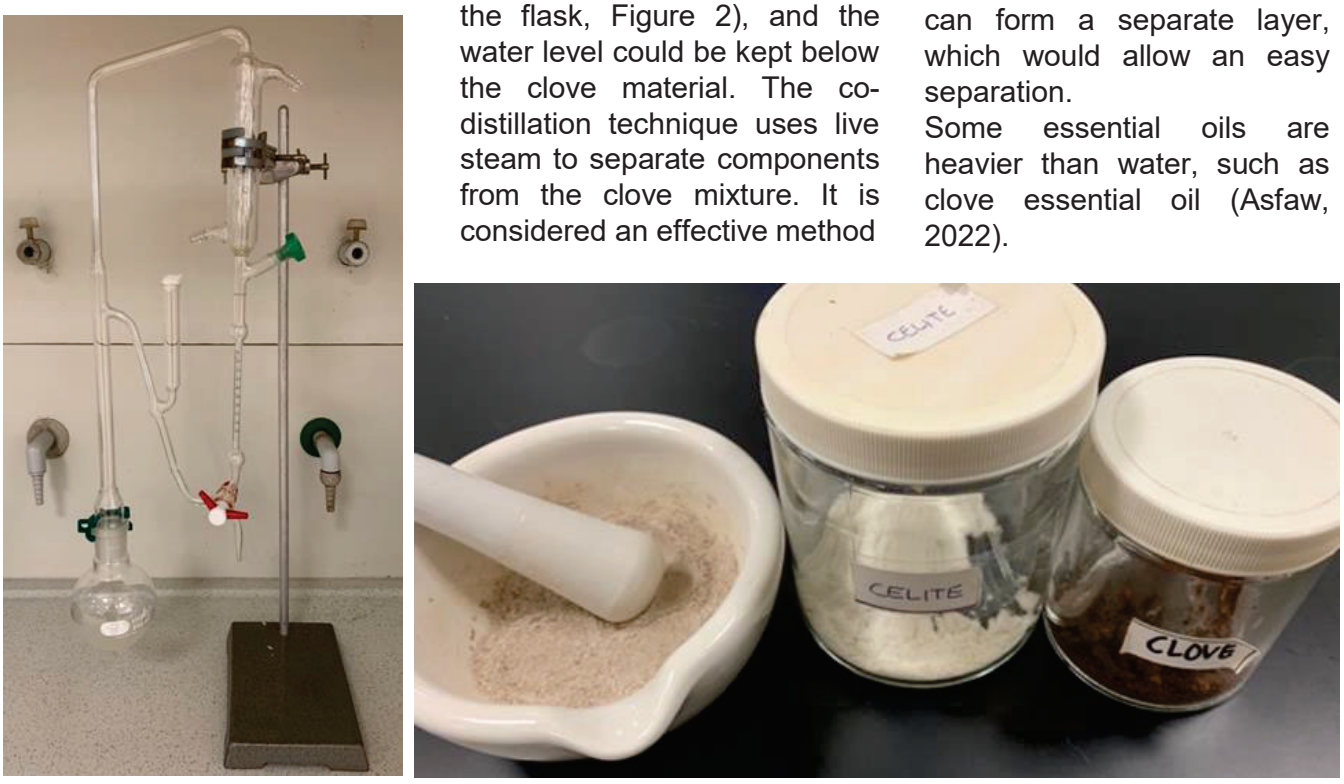
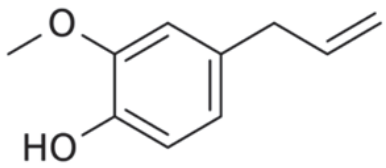
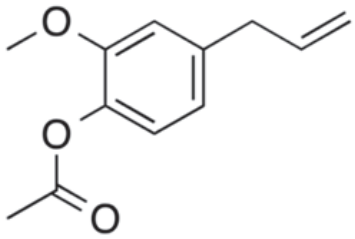


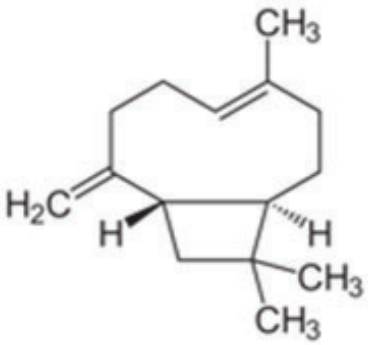
Figure 2: The Clevenger or Dean-Stark apparatus is set for the pharmacy undergraduates for their laboratory practical (left). The dried cloves were mixed with Celite® in a mortar, to make a homogenous powder prior to the distillation (right) (Source: authors' own collection)



The chemical structure of eugenol ($C_{10}H_{12}O_2$), bp = 254 ^{0}C, or 4-allyl-2-methoxyphenol or 2-methoxy-4-(prop-2-en-1-yl)phenol, is the characteristic aroma (principal constituent: 60 - 90% of distilled clove bud oil



Acetyl eugenol ($C_{12}H_{14}O_3$) or eugenol acetate or (2-methoxy-4-prop-2-enylphenyl) acetate (10 - 30% of distilled clove bud oil)



Caryophyllene or β-caryophyllene ($C_{15}H_{24}$), a natural bicyclic sesquiterpene of distilled clove bud oil, which is composed of three units of isoprene.

Figure 3: The natural constituents in the essential oil from the clove flower bud (Source: authors' own collection)

They can be found at the bottom of the extraction flask. Once the essential oil is evaporated and the vapor condenses after passing the condenser, it could float on top of the water level and can be trapped with xylene. Another alternative to this traditional steam distillation for the extraction of clove oil would be a modern, household espresso machine, as reported earlier by Just et al. (2016).

In the practical session, the students were asked to grind about five grams of clove (Figure 2), with five grams of diatomaceous earth (Celite®) to make a fine, homogenous powder and form an anti-bumping mixture. After that, they were instructed to use four grams of that mixture and add about a hundred ml of distilled water in a 250 ml round bottom flask. The students are reminded to carefully clamp and clip the glassware before the heating step. The volatile oil in the clove sample could be determined. The presence of eugenol ($C_{10}H_{12}O_2$, Figure 3) could be detected in the clove oil. Eugenol, in fact, can be obtained from a wide range of plant sources including nutmeg oil and cinnamon extract. Eugenol has strong health-promoting functions that make it a versatile natural ingredient. It is a substituted methoxyphenol, with an allylic functional group in the para position. The allylic chain gives eugenol its characteristic strong odour. This phenolic compound is declared as GRAS (generally recognized as safe) by the World Health Organization (WHO) and is considered as non-mutagenic (Ahmed Khalil et al. 2017). Numerous investigations documented the therapeutic potential of eugenol highlights its importance as one of the principal bioactive components, having several health- promoting functions. These would include eugenol as having antioxidant, antimicrobial and anti-cancer activities. Reports were also published on the anti-diabetic and anti-inflammatory potentials of this phenylpropanoid class of compound. The practical procedures could be run in about three hours, with moderate difficulty, since this might be the first moment the students would handle the equipment and glassware meant for the Clevenger apparatus. Furthermore, the materials are also comprised of chromatographic experiments, to observe the components in the clove oil (Silver, 2020; GlobinMed, 2022; Ciaccio et al. 2022). The total ion chromatogram of *S. aromaticum* oil was also recorded (Rodríguez et al. 2014). The quantification of eugenol as the principal component, was obtained by gas chromatography-mass spectrometry. In addition to the above compounds in Figure 3, other aromatic components such as vanillin and thymol, were also isolated from the clove oil.

References