Antidepressant activity of patchouli alcohol microcapsule

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Abstract. Patchouli alcohol is an essential oil compound which obtained by fractional distillation of patchouli oil (Pogostemon cablin Benth). Patchouli alcohol has several benefits in aromatherapy field. Patchouli alcohol is useful for reduce depression by breathing is steam. The essential oil is a sensitive and susceptible material towards high temperature, oxidation, UV light, and humidity which are able to cause the oxidative damage. Encapsulation can be the way to resolve this problem. Maltodextrin and gum arabic are kind of good catalysts in the essential oil encapsulation. In this research will be done patchouli alcohol encapsulation by using freeze drying method with maltodextrin, gum arabic and tween 80 catalysts as emulsifier. Patchouli alcohol that will be used is coming from the distillation with the content of 42.37% which will be tested its antidepressant to mice further. The test results of the best antidepressant was on microcapsule powder with the variation of (1 g maltodextrin: 1 g gum arabic: 3 g patchouli alcohol).

1. Introduction

Depression is a psychiatric syndrome whose manifestations can be a feeling of moodiness, weight loss, psychomotor decline, and loss of life passion [1]. More than 121 million people worldwide are thought to suffer from depression [2]. The tendency of humans to experience depression causes many people to need an alternative therapy to improve their emotional level to improve their mood or mood. In this case aromatherapy appears as an alternative to overcome depression. Aromatherapy is said to be an antidepressant if it is able to activate psychomotor so that it can reduce and eliminate feelings of moodiness and can improve mood. Antidepressant activities of Citrus aurantium L. var., Amara Ocimum gratissimum L., almond oil, and Rosmarinus officinalis L, essential oil were investigated [3-6].

Patchouli oil (Pogostemon cablin Benth) is widely used as aromatherapy to overcome depression. The test of aromatherapy of patchouli oil and cananga oil as an antidepressant for the motor activity of mice by using ultrasonic sound waves of 30.000 Hz as inducers of depression [7]. The study explained that only 1% patchouli oil can be used as aromatherapy for antidepressants for mice. Essential oil is a sensitive material and is susceptible to high temperatures, oxidation, UV light and moisture so that a way to overcome these problems is needed. Encapsulation can provide a solution to the problem. Encapsulation is a encapsulate technology that is widely used to keep essential oils protected from degradation problems and can release their contents under certain conditions. Nanoparticle complexes of geraniol and β-cyclodextrin were successfully prepared using co-precipitation. The encapsulation efficiency of the NP complexes was optimized at different ratios of geraniol to β-cyclodextrin [8]. In the encapsulation process, the thing to note is the type of encapsulate. In this study encapsulation of patchouli alcohol will be carried out using a maltodextrin and gum arabic encapsulate which will then
be tested as an antidepressant. The aim of the study was to determine the effect of a mixture of patchouli, maltodextrin and gum arabic on the characteristics of microcapsules and to know the activity of patchouli alcohol microcapsules as antidepressants.

2. Methods
The equipment used includes: a set of fractionation distillation reduced pressure, ultrasonic with a frequency of 3000 Hz, a box antidepressant tester with a length of 55 cm, width of 35 cm, and height of 30 cm, and Gas Chromatography (GC) Agilent Cerity, Gas Chromatography Mass Spectrometry (GC-MS) GCMS QP2010S Shimadzu, Agilent HP IMS column pressurized helium carrier gas 13.7 kPa, helium flow rate 40 mL / minute, program column temperature 70°C to 300°C and the Perkin Elmer Spectrum Version Fourier Transformed Infrared Spectroscopy (FT-IR) 10.03.06. The materials used were patchouli oil from Lansida Yogyakarta, maltodextrin, gum arabic, tween 80, aquademin, ethanol, n-hexane, white male mice (Mus musculus) from the Biology Laboratory of Universitas Negeri Semarang.

Compounds in patchouli oil was identified by GC-MS analysis. Isolation of patchouli alcohol from patchouli oil was done by fractional distillation under reducing pressure. As much as 200 mL of patchouli oil are put into a 500 mL boiling flask, then performed fractionation distillation with reduced pressure. Fraction that contain digest of patchouli alcohol then redistilled by fractionational distillation under reducing pressure to obtain higher concentration of patchouli alcohol.

Encapsulation was done by dispersion of patchouli alcohol in encapsulate emulsion (maltodextrin and gum arabic in a mixture of 70% ethanol, water and tween 80. The mixture of encapsulate and solvent heated to 55°C while stirring at 500 rpm, then 3 g of patchouli alcohol added in the 5 g of encapsulate dispersion, after the dispersion was completed stirring is carried out for 4 hours, then the solution cooled in the freezer is then freeze-dried using a freeze dryer to convert patchouli alcohol slurry to encapsulated patchouli alcohol powder. The ratio between encapsulate and patchouli alcohol was 2:3; 5:3; and 5:5 g/g in 25 mL of solvent. The encapsulation process begins by making a suspension between patchouli alcohol, encapsulate and solvent then stirring at a speed of 500 rpm to make it homogeneous and forming a bond between patchouli alcohol and a mixture of maltodextrin and gum arabic until patchouli can be coated well and the results are not volatile. The suspension is then frozen in the freezer at ±-80°C for 4 hours and vacuumed using a freeze dryer (FD-1 EYELA) for 32 hours to remove the solvent to form a dry powder. In the freeze drying process, the suspension of the overlaid, encapsulate and solvents was frozen at ±80°C, after the suspension became frozen cold vapor was emptied with a vacuum pump to increase sublimation energy and dry the suspension into powder.

**Table 1. Variation in suspension of patchouli alcohol encapsulation**

| Code | Maltodextrin (g) | Gum arabic (g) | Patchouli alcohol (g) |
|------|-----------------|----------------|----------------------|
| A1   | 1               | 1              | 3                    |
| A2   | 2               | 3              | 3                    |
| A3   | 3               | 2              | 5                    |

The powder was analyzed by controlled release by using GC. Measurements were determined in a range of 0, 2, 4, 6 and 8 days. Encapsulated patchouli alcohol was placed at room temperature (± 27 °C) in the desiccator below which is given silica gel 0.2 g of patchouli alcohol capsules were dissolved in 2.5 mL n-hexane then vortexed the results were injected into GC. The concentration of compounds in capsules is obtained by comparing the area of each compound with the total area.

Antidepressant tests was measured by modified Betharani et al. method [7], 0.2 g of patchouli alcohol capsules was taken and placed in a container. Mice were divided into 3 groups, namely the control group which was only given powder encapsulate in the form of maltodextrin and gum arabic, the test group with a ratio of 2: 3, 5: 3 and 5: 5. All mice are left alone for 5 minutes in the test box to adapt. Then, testing depression begins when the ultrasonic is turned on for 30 minutes. After a while, these mice will move passively even in silence, this shows despair as a sign of depression. Then after the mice have
been depressed, antidepressants are inserted into the test box. Observations began after administration of antidepressants, then observed and measured the length of the mice moving passively for 15 minutes at 5-minute intervals. All data were processed using the independent t test method, then graphs were made showing the relationship of treatment with the length of time the mice remained silent.

3. Results and Discussion

3.1. Isolation of Patchouli alcohol

Identification of patchouli oil components was comparing the mass spectra of each component identified with the literature. The chemical composition of patchouli oil from the analysis results was shown in Figure 1 and Table 2.

![Chomatogram of Patchouli oil](image1)

**Figure 1. Chromatogram of Patchouli oil**

| No. | Component            | RT (minute) | Area (%) |
|-----|----------------------|-------------|----------|
| 1   | α-Gurjunene          | 20.4        | 2.87     |
| 2   | β-Elemene            | 20.7        | 1.42     |
| 3   | *trans*-Caryophyllene| 21.5        | 4.46     |
| 4   | α-guaiene            | 22.0        | 18.78    |
| 5   | Seychellene          | 22.1        | 5.31     |
| 6   | α-Patchouline        | 22.5        | 8.58     |
| 7   | Alloaromadendrene    | 23.5        | 4.09     |
| 8   | Δ-Guaiene            | 23.7        | 21.60    |
| 9   | Patchouli alkohol    | 27.6        | 32.88    |

Based on data in Table 1 it can be seen that the chemical components of patchouli oil consist of oxygenated sesquiterpenes and hydrocarbon sesquiterpenes and patchouli alcohol is the largest component in patchouli oil (32.88%). The differences in the chemical components that make up patchouli oil qualitatively and quantitatively can be caused by several factors including the difference in environmental factors from the origin of patchouli oil. The mass spectrum of peak 9 shows molecular ion peak identified at m/e 222. The fragmentation shows peaks at m/e 207 (M+-15), 189, 179 (M+-43), 161, 138, 125, 95 and base peak at m/e 41. The base peak at m/e 41 is suggested from the release of isopropyl at m/e 189 which produce fragment with m/e 43 and then releases H₂ to form based peak fragment at m/e 41.
The results of fractional distillation and redistillation of 200.0 mL of patchouli oil was obtained 20.0 mL of yellow distillate with woody and stinging odor. The fraction was then analyzed for purity using gas chromatography, the chromatogram showed that that patchouli alcohol concentration increased to 42.37% at retention time of 26.22 minutes. (Figure 3).

3.2. Encapsulation of Patchouli Alcohol

The results of patchouli alcohol encapsulations were white powder and have a smell of patchouli alcohol. A1 microcapsules was a brownish-yellow powder and lumpy, this can occur because the amount of encapsulate is less than the amount of patchouli alcohol which causes the core material cannot be coated completely. A2 microcapsules was a brownish-yellow powder but does not clot, this brownish color is due to more gum arabic encapsulate than maltodextrin. In this case maltodextrin has low browning properties when forming a matrix. A3 microcapsules was white powder, does not clot, and smells patchouli alcohol. A3 microcapsules are the best powder, because the ratio of encapsulate and patchouli alcohol is right, 5: 5. The amount of maltodextrin encapsulate is greater than that of gum arabic causing white powder. The best encapsulation results were then analyzed using the infrared spectrum to find out that there were still OH groups in patchouli alcohol after encapsulation, which can be presented in Figure 4 below.
Figure 4. IR spectrum of microcapsule A3

Infra red spectrum in Figure 4 shows the peak of -OH group at wave number 3394.43 cm\(^{-1}\) is a characteristic of compounds that have hydrogen bonds, CH bands appear from \(-\text{CH}_2\) and \(-\text{CH}_3\) bands at wave number 2928.88 cm\(^{-1}\), and the \(-\text{CH}_2\) and \(-\text{CH}_3\) groups of waves appear in 1458.86 and 1375.71 cm\(^{-1}\), and the \(-\text{CO}\) groups from C-OH appear at 1083.43 and 1039.54 cm\(^{-1}\).

3.3. Antidepressant activity

The antidepressant activity test was carried out using an ultrasonic device with a frequency of 3000 Hz. The selection of the 3000 Hz frequency is because mice have a hearing range that is below 35,000 Hz. The mice used in this study never been used in previous studies. This stage aims to find out how far the activity of antidepressant powder against mice. The principle of antidepressant test is to determine immobility duration of mice after treatment with antidepressant powder. Twelve mice were divided into 4 groups.

| Groups | Code | Composition |
|--------|------|-------------|
| Control | A  | 1 | 1 | - |
| 1 | A1 | 1 | 1 | 3 |
| 2 | A2 | 2 | 3 | 3 |
| 3 | A3 | 3 | 2 | 5 |

Based on the results of the antidepressant test, it can be seen that the longer the treatment time in mice, the less time that mice experience. The response to the smell of patchouli alcohol inhaled by mice can cause a sense of calm that will stimulate areas in the brain so that depression decreases. Depression syndrome is caused by insufficiency in the activity of several neurotransmitters such as noradrenaline, serotonin, and dopamine in the limbic system. The limbic system in the brain is a place of storage of memory, regulation of mood, emotions, personality, sexual orientation and can affect behavior [7]. The antidepressant test results can also be obtained by the percentage of depression reduction in each test group. A1 test group produced 76.33% depression reduction percentage, A2 group produced 15.15%, and A3 group produced 14.61%. Percentage reduction in depression indicates the ability of antidepressant powder to reduce depression. The ideal value for the percentage reduction in depression is 50%.
Table 4. Duration immobilization of mice in antidepressant test

| Treatment | Mice | Duration of immobilization (sec) |
|-----------|------|----------------------------------|
|           |      | 5’ | 10’ | 15’ |
| A         | 1    | 74 | 199 | 161 |
|           | 2    | 220| 166 | 276 |
|           | 3    | 197| 271 | 300 |
| Total     |      | 461| 636 | 737 |
| Average   |      | 154| 212 | 246 |
| A1        | 1    | 112| 76  | 4   |
|           | 2    | 39 | 33  | 45  |
|           | 3    | 88 | 34  | 3   |
| Total     |      | 239| 143 | 52  |
| Average   |      | 80 | 48  | 17  |
| A2        | 1    | 243| 202 | 267 |
|           | 2    | 165| 130 | 70  |
|           | 3    | 229| 188 | 62  |
| Total     |      | 637| 520 | 399 |
| Average   |      | 212| 173 | 133 |
| A3        | 1    | 3  | 117 | 93  |
|           | 2    | 183| 245 | 261 |
|           | 3    | 160| 245 | 259 |
| Total     |      | 346| 607 | 613 |
| Average   |      | 115| 202 | 204 |

4. Conclusion

The best characteristics of patchouli alcohol microcapsules were obtained from a dispersion of 5 g patchouli alcohol which was coated in a mixture of maltodextrin and gum arabic (3: 2) with the same mass. Antidepressant activity is affected by the amount of coated patchouli alcohol.

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