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Original Article

Essential Oil of Alpinia galangal: Effect of Aromatherapy Inhalation in Mice and Physicochemical Characterization

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Abstract: Aromatherapy has been used for centuries to maintain body and mental health. In aromatherapy, the inhalation of essential oil provides physical and psychological benefit due to its volatile bioactive compounds. The current study was aimed to determine the effect of the inhalation of *Alpinia galanga* essential oil in mice using forced swim test. The refractive index, solubility, and Gas Chromatography-Mass Spectrophotometry (GC-MS) profile of AGEO were also investigated. The essential oil from *Alpinia galanga* showed an anti-depressive-like effect and it was as strong as the positive control (*Lavandula angustifolia* essential oil). The GC-MS analysis discovered some antidepressant compounds in *A. galanga* essential oil such as eucalyptol, fenchone, and α-terpineol.

Keywords: *Alpinia galanga*, antidepressant, aromatherapy, essential oil, physicochemical characterization

INTRODUCTION

Aromatherapy is defined as the use of essential oils, through inhalation or direct application to the skin, to achieve physical and spiritual well-being. It has been used for centuries in traditional practice to manage various symptoms including symptoms of mental disorder such as depression (Williams, 2015). According to Bueno-Notivol et al., (2021), the prevalence of depression increased from 3.44% in 2017 to 25% in 2020 due to the pandemic of COVID-19. Patients with depression usually consume chemical drugs for long-term therapy. On the other hand, conventional antidepressants cause many adverse effects (Sohrabi et al., 2017). Thus, it is important to prove the efficacy of using essential oil in aromatherapy to relieve mental disorder.

Many studies have been conducted to explore the antidepressant-like effect of essential oils. Some of them were using single essential oil such as lavender, neroli, and lemon essential oils, also combined-essential oils for example Anshen essential oil (mixture of lavender, sandalwood, sweet orange, frankincense, rose, orange blossom, and agarwood oil) (Chioca et al., 2013; Dosoky & Setzer, 2018a; Zhong et al., 2019).

In this study, we utilized the essential oil of *Alpinia galanga* (Zingiberaceae). The rhizome of *A. galanga* is used by the Indonesian people as a cooking spice and traditional medicine. It has been explored to show many pharmacological activities including antitumor, antidiabetic, immunomodulator, antioxidant, anti-inflammation, antiproliferative, apoptotic, antiangiogenic, antimicrobial, antifungal, and anti-allergic activities (Chouni & Paul, 2018; Khairullah et al., 2020).

On the other hand, there is no report related to the potency of the inhalation of its essential oil to reduce depressive behavior. Therefore, we aimed to evaluate the anti-depressive-like effect of the inhalation of *A. galanga* essential oil using a forced swim test and determine its physicochemical characteristic through the refractive index, solubility, and GC-MS analysis. The result of this study was expected to give scientific evidence to the aromatherapy of *A. galanga* essential oil.

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METHOD

Tools and Materials.

Tools. The tools used in the current study were the Atago NAR-1T Solid refractometer and Gas Chromatography - Mass Spectrophotometry (Agilent Technologies).

Materials. Essential oils of *A. galanga* was purchased from Gallery Essential Oil & More (Denpasar, Bali). Essential oil of *L. Angustifolia* was purchased from Rumah Atsiri (Karanganyar, Central Java). Other materials were 96% ethanol (Merck; Darmstadt, Germany) and aquadest.

Refractive index measurement

The refractive index of *A. galanga* and *L. angustifolia* essential oils was estimated based on a method described by Karakaya *et al.* (2012) with slight modification. Essential oil 200 µL was put into the Atago NAR-1T Solid refractometer. The measurement temperature was 20°C.

Solubility measurement

The solubility of *A. galanga* and *L. angustifolia* essential oils in 96% ethanol was determined with the procedures from another publication (Anwar et al., 2011). A test tube was filled with 1 mL of essential oil, followed by 96% ethanol until a clear solution was achieved.

Gas Chromatography-Mass-Spectrometry (GC-MS) analysis

The volatile compounds of *A. galanga* and *L. angustifolia* essential oils were detected using an Agilent Technologies 7890B GC, 5977B MSD fitted with HP-5MS UI column (30 m length, 0.25 mm film thickness, and 0.25 m internal diameter). The temperature was raised at a rate of 10°C/minute from 70°C to 290°C.

Forced swim test

The procedure was modified from another studies (Abbasi-Maleki et al., 2020). Two until three months old male mice weighing 20 grams were adapted for 7 days. They were then randomly divided into 3 groups (negative control (aquadest), positive control (*Lavender angustifolia* essential oil), and *A. galanga* essential oil (AGEO) groups). Furthermore, essential oils were diluted with aquadest until 1% and put into a diffuser. The diffuser was put into a test room, turned on for 15 minutes, until the test room be saturated with essential oil vapor. After that, the animal model was put into the test room, inhaled the essential oil, and then moved to a cylinder (10 x 25 cm) filled with water. At first, the animal will try to escape but eventually will exhibit immobility. The period when the mice tried to float on water without struggling and only tried to maintain their head above the water is recorded as immobility time.

Ethical clearance

The Animal Ethics Committees, Faculty of Veterinary Science, Udayana University (Certificate Number: B/121/UN14.2.9/PT.01.04/2021) authorized the procedure used in this study.

Data analysis.

The antidepressant-like effect was stated as median immobility time (s), with minimum and maximum values in parentheses. Data were assessed by the non-parametric Kruskal Wallis test, followed by the Mann Whitney post hoc test with SPSS 24 for windows. The P values less than 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

The essential oil of *A. galanga* is presented in its leaves, stems, rhizomes and roots. The oil contained mono- and sesquiterpenes (Chouni & Paul, 2018). The current study determined the physical properties of *A. galanga* essential oil (Table 1). Physicochemical characterization of essential oil like color, refractive index, solubility, and GC-MS analysis indirectly informs about the quality of the oil (Barkatullah et al., 2012). It is normally for essential oils to have the refractive index from 1.450 to 1.590 (Baser & Buchbauer, 2016).

Table 1. The physical properties of *Alpinia galanga* and *Lavender angustifolia* essential oil

Physical properties	AGEO	LAEO
Physical color and appearance	Liquid, yellow	Liquid, yellow
Refractive index (20°C)	1.553	1.457
Solubility	soluble in 2 mL of 96% ethanol	soluble in 2 mL of 96% ethanol

AGEO = *Alpinia galanga* essential oil, LAEO = *Lavandula angustifolia* essential oil

The volatile compounds of *A. galanga* essential oil were detected by GC-MS analysis (Table 2). There were 6 compounds detected which 3 of them were classified as monoterpenoid (eucalyptol, fenchone, and α-Terpineol). A review reported that the *A. galanga* oil contained other main volatile compounds such as Galangin, α-fenchyl acetate, β-farnesene, β-bisabolene, α-bergamotene, β-pinene, 1'S-1'-acetoxychavicol acetate, 1'S-1'acetoxyeuginol acetate, β-Sitosteroldiglucoside (AG-7), β-sitsteryl-Arabinoside (AG-8), 1'-acetoxychavicol acetate (galangal acetate), p- droxycinnamaldehyde, and [di-(p-hydroxy-cis-styryl)] methane (Chouni & Paul, 2018). A GC-MS analysis by Wu et al., (2014) showed the major components of *A. galanga* oil were eucalyptol, (1S)-(1)-β-pinene, 1R-α-pinene, α-terpineol and L(-)-borneol. Variations on GC-MS analysis might due to intrinsic and extrinsic aspects such as different sources of botanical origin (Dhifi et al., 2016). The volatile compounds of the positive control for the forced swim test, *L. angustifolia* essential oil, were also evaluated (Table 3). The oil contained antidepressants such as myrcene, limonene, α-Terpineol, linalool, and germacrene (Guzmán-Gutiérrez et al., 2015; Khaleel et al., 2018; Setzer, 2009).

Table 2. The volatile compounds of *Alpinia galanga* essential oil by GC-MS analysis

No.	Volatile compounds
1	Eucalyptol
2	Fenchone
3	α-Terpineol
4	Triacetin
5	2-Propenoic acid, 3-phenyl-, methyl ester
6	Fumaric acid, 3-methylbutyl 2-chloro-6-fluorphenyl ester

Table 3. The volatile compounds of *Lavender angustifolia* essential oil by GC-MS analysis

No.	Volatile compounds
1	β-Myrcene
2	D-Limonene
3	Eucalyptol
4	β-Ocimene
5	Linalool
6	1-Octen-3-yl-acetate
7	(+)-2-Bornanone
8	Camphor
9	Endo-Borneol
10	Terpinen-4-ol
11	α-Terpineol
12	Lavandulyl propionate
13	Neryl (S)-2-metylbutanoate
14	2,6-Octadien-1-ol-,37-methyl-acetate
15	(E) -β-Femesene
16	Germacrene
17	Caryophyllene oxide

In the current study, the forced swim test showed that the immobility time of the *A. galanga* essential oil group was significantly lower than the negative control (Table 4), indicating its potential to reduce depressive behavior. Meanwhile, it was not significantly different from the positive control group which signified an equal strength. The similar results from forced swim test have also been observed in other studies with various essential oils such as *Asarum heterotropoides*, *Zingiber officinale* oil, *Thymus vulgaris*, *Mentha x piperita*, *Cupressus sempervirens*, and *Hypericum scabrum* (Mohammed Sur et al., 2019; Park et al., 2015; Won et al., 2005).

The medicinal properties of essential oils in aromatherapy is due to the presence of bioactive compounds, such as monoterpenes, sesquiterpenes, and aromatic compounds (Zhang et al., 2021). Based on literatures study, the possible bioactive compounds in *A. galanga* essential oil to produce antidepressant-like effect were eucalyptol, fenchone, and α-Terpineol. The antidepressant-like activity of the inhalation of eucalyptol or 1,8 cineole was studied by (Dougnon & Ito, 2020). In the forced swim test, 1,8-cineole at doses of 4×10^{-4} and 4×10^{-2} mg induced a significant decrease in the immobility time of mice by 44% and 39%, respectively.

Fenchone is classified as monoterpenoid with a ketone functional group. It was also found in essential oils from other plants such as *Apium graveolans*, *Citrus reticulata*, and *Foeniculum vulgare* (De-Montijo-Prieto et al., 2021). Ganesh et al. (2019) reported its antidepressant like activity in rodents with chronic unpredictable mild stress. At doses of 400 mg/kg and 800 mg/kg given orally, fenchone could relieve the depression.

Table 4. Immobility time (s) and P values from the Mann Whitney post hoc test from tested groups

Experimental Group	Median of IM (min-max)	P values		
		AGEO	Negative control	Positive control (LAEO)
AGEO	29.5 (23-40)	-	0.004*	0.127
Negative control	65 (61-99)	0.004*	-	0.004*
Positive control (LAEO)	24.5 (16-33)	0.127	0.004*	-

IM = Immobility time (s), AGEO = *Alpinia galanga* essential oil, LAEO = *Lavandula angustifolia* essential oil, *significant difference (p<0.05)

Alpha-terpineol was also detected in essential oils from other plants, including *Plumeria alba*, *Kaempferia daklakensis*, *Alpinia calcarata, Curcuma aromatica*, *Citrus spp*. (Dosoky & Setzer, 2018a, 2018b; Lawal et al., 2014; Tuan et al., 2019). It was the main component of *Oreganum majorana* (Abbasi-Maleki et al., 2020). Assunção et al. (2021) studied the effectiveness of alpha-terpineol to reduce depression through behavioral tests, which were the forced swim test and tail suspension test. The study showed that alpha-terpineol at doses 50 mg/kg and 100 mg/kg given orally demonstrated a lower immobility time than control groups which indicated its potency as an antidepressant agent.

CONCLUSION

Aromatherapy inhalation of Alpinia galanga essential oil showed anti-depressive-like effect. The anti-depressive-like effect of *Alpinia galanga* essential oil was similar to positive control (*Lavendula angustifolia* essential oil). The essential oil of Alpinia galanga contained antidepressants such as eucalyptol, fenchone, and α-terpineol.

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