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Composition of Compound 1.8-cineole from *Eucalyptus pellita* Leaf Extract Using 2 Types of Solvents and Phytotoxicity Against *Borreria alata* (Aubl) DC Weeds

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Abstract. The monoterpene group has a molecule called 1.8-cineole as its primary component. The compound 1.8-cineole is one of the most powerful allelochemicals released by many species, such as Eucalyptus spp., which is toxic to other plants. This study intends to identify a suitable solvent to extract 1.8-cineole derived from Eucalyptus pellita leaf extract using 2 types of solvents, ethanol and aquadest along with their phytotoxicity properties. This research was conducted in 2 stages. The first stage involved the extraction of Eucalyptus pellita leaves using the Soxhlet method, and the extraction results were analyzed with GC/MS. The second stage of the phytotoxicity test against *Borreria alata* (Aubl) DC., weeds used seven levels of extraction formula treatment. The extraction results using ethanol solvents showed higher yields of 1.8-cineole compound composition compared to using aquadest solvents. The results of the phytotoxicity test of variance showed a significant difference in the wet and dried mass of weeds. The extraction formula with a concentration of 50% *Eucalyptus pellita* leaf extract using ethanol as a solvent has the best phytotoxicity properties compared to other extraction formulas.

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1. Introduction

Eucalyptus pellita is known for its allelopathic properties, where certain chemicals it produces can inhibit the growth of other plants. Khalid et al. (2021) demonstrated that extracts from Eucalyptus camaldulensis and *Citrus aurantium* significantly reduced the radicle and plumule lengths of *Triticum aestivum*. Audina's research (2017) further highlighted the potential of *E. pellita* leaves as post-emergence bioherbicides, showing their effectiveness in suppressing the growth of weeds like *Asystasia intrusa, Borreria alata,* and *Cyperus brevifolius*, which led to a reduction in weed leaf counts.

The primary chemical components of *E. pellita* leaves are monoterpenes, with 1.8-cineole being the dominant compound (Ghasemi et al., 2018; Kordali et al., 2016). To isolate 1.8-cineole, a solvent with similar polarity is required. In their study, Jaime et al. (2017) obtained a 76.43% yield of 1.8-cineole from *Eucalyptus globulus* using ethanol. Comparatively, Irvan et al. (2015) and Andriyani (2017) achieved lower yields—29.17% and 26.95% respectively—when using dichloromethane and distilled water (aquadest) as solvents to extract 1.8-cineole from *Eucalyptus urophylla*.

A comparative study is necessary to assess the efficacy of ethanol and distilled water as solvents for extracting 1.8-cineole from *E. pellita* leaves. This research aims to determine the most effective solvent for extracting this compound while evaluating its phytotoxic effects on weeds. Ethanol and distilled water were tested as solvents to explore their phytotoxicity and extractive potential of 1.8-cineole from *E. pellita* leaves.

2. The Methods

2.1. Preparation of powdered *E. pellita* leaves

The freshly harvested *E. pellita* leaves were meticulously rinsed with clean running water. Subsequently, the leaves were allowed to dry naturally for a period of 7 days. After the drying process, the leaves were pulverized using a grinder equipped with a 40 mesh sieve. The resulting powder was weighed up to 15 g and then combined with 300 ml of either aquadest or ethanol in a ratio of 15 g: 300 ml.

2.2. Producing an extract from *E. pellita* leaves

Solvent extraction of *E. pellita* leaves was performed using the soxhletation technique with 96% ethanol and aquadest at a temperature of 80°C. Pulverised *E. pellita* leaf powder weighing up to 15 g was enclosed in Whatman filter paper no. 40. To obtain the extraction solution, the weighted leaf powder is placed into a soxhlet tube and 300 ml of 96% ethanol solvent is added to the solvent in the soxhlet extractor operating at a temperature of 80°C for 8 hours. The extraction solution is introduced into a rotating water bath tube to undergo a purification procedure in order to accomplish the separation of ethanol from specific components, including the 1.8-cineole compound. The following step involves diluting the solution with distilled water to achieve the desired concentration (Irvan et al., 2015).

2.3. Characterization of the 1.8-cineole chemical composition

Analysis of *E. pellita* leaf extraction using ethanol and distilled water solvents was conducted using GC/MS to quantify the total component content.

2.4. Evaluation of phytotoxicity

Phytotoxicity testing was conducted on *B. alata* (Aubl) DC. weeds sown in 30cm × 40cm polybags containing planting media consisting of soil mixed with cocopeat in a 3:1 ratio. Five potato weeds were selected from HTI plantations, placed in each polybag, and watered until fully saturated. Chosen weeds that have reached 7 days of age after planting, while ensuring that three plants have a similar height and leaf count among each other. The application of bioherbicide up to 20 ml per treatment was conducted 2 weeks after planting (Rokhmaningsih, 2018).

2.5. Design of the study

The study employed a completely randomized design (CRD) 1 factor method with 7 levels of extraction formula treatment. Trial 1: Control (applied with distilled water spray); Concentrations of Eucalyptus leaf extract in ethanol solvents are as follows: T2: 25% Eucalyptus leaf extract; T3: 50% Eucalyptus leaf extract; T4: 75% Eucalyptus leaf extract; T5: 25% Eucalyptus leaf extract; T6: 50% Eucalyptus leaf extract;

T7: 75% Eucalyptus leaf extract. Each treatment was replicated four times, resulting in a total of 28 experimental units.

2.6. Observation of wet and dried bulk mass of weeds

At the conclusion of the observation or 21 days after application, the wet and dried mass of weeds was measured, beginning from the roots (bottom of the weed) and extended to the stems and leaves (top of the weed). Measure the mass of the weeds in their fresh state (after being removed from soil, garbage, or other non-weed components), then subject them to a 48-hour drying process in an oven set at a temperature of 65°C. Finally, weigh the dried mass of the weeds using an analytical scale.

2.7. Analysis of data

In order to ascertain the impact of therapy, the observation data were subjected to analysis of variance (ANOVA) at a significance level of 5%. The statistical analysis of variance which had a significant impact on all observation parameters was subsequently evaluated using Duncan's Multiple Range Test (DMRT) at a significance level of 5% using the SPSS-25 software.

3. Result and Discussion

3.1. Chemical composition of the 1.8-cineole compound

Analyzing the chemical makeup of the 1.8-cineole component from *E. pellita* leaf extract was conducted by utilizing various solvents to determine the most suitable solvent for the extraction procedure. The chemical makeup of the 1.8-cineole molecule derived from the leaf extract of Eucalyptus pellita used ethanol and aquadest solvents is presented in Table 1.

Table 1. Composition of the compound 1.8-cineole in *E. pellita* leaf extract.

Solvent	Area	Concentration (%)	Diluting for 4 times
Ethanol	0.600875495	4.43	17.74
Aqudest	0.052502248	0.55	2.19

The data shown in Table 1 indicates that the extract obtained using ethanol solvent had a greater concentration of the 1.8-cineole component, namely 4.43%, in comparison to the extract obtained using aquadest solvent, which stood at 0.55%. Owing to its greater polarity index than aquadest, ethanol is classified as a polar organic solvent. According to Irving et al. (2015), the outcomes obtained in the extraction process are influenced by the chemical polarity of the solvent. In comparison to aquadest, ethanol as a solvent has a lower electrical constant, which falls within the range of 33 to 88. The dielectric constant is the repulsive force that acts between two electrically charged particles within a crystal structure. A higher dielectric constant indicates a greater polarity of the solvent. This indicates that the ethanol solvent may effectively extract chemicals from *E. pellita* leaves, as the interaction between the compounds and the solvent depends on their similar polarity (Verdiana et al., 2018).

3.2. Assessment of phytotoxicity against *B. alata* (Aubl) DC weeds

The analysis of variance on the parameters of wet and dried mass of weeds revealed that the application of Eucalyptus pellita leaf extract had a statistically significant impact on the wet and dried mass of Borreria alata (Aubl) DC. weeds, 21 days post exposure. The outcomes of the 5% dimethyl ether reduction test are displayed in Table 2.

Table 2 clearly demonstrates that the wet and dried mass of *B. alata* (Aubl) DC. weeds are greatly affected by the specific solvent and concentration employed. Significant variations were seen in the wet and dried mass of weeds 21 days post-application when using ethanol solvents containing 50% and 75% *E. pellita* leaf extract across all treatments. The most effective treatment for reducing the wet and dried

mass of weeds was identified as a 75% extract with ethanol solvent, resulting in a wet mass of 7.4g and a dried mass of 2.5g. This treatment was not substantially different from the 50% extract with ethanol solvent.

Table 2. Wet and dried mass of *B. alata* (Aubl) DC. weeds, 21 days after application of *E. pellita* leaf extract.

Treatment	Wet mass (g)	Dried mass (g)
Control (spray with aquadest)	48.0 a	7.5 a
25% extract with ethanol	38.5 ab	5.4 ab
50% extract with ethanol	10.1 c	3.1 c
75% extract with ethanol	7.4 c	2.5 c
25% extract with aquadest	38.9 ab	5.6 b
50% extract with aquadest	36.7 b	5.0 b
75% extract with aquadest	36.5 b	4.9 b

Note: the value followed by the same lowercase letter in the same column reveal no significant changes according to the 5% DMRT test.

The study undertaken by Andriyani (2017) yielded same findings with regards to the impact of administering *E. pellita* leaf extract at concentrations of 30% and 40% on the dried mass of Borreria alata weeds. The study conducted by Naema et al. (2016) found that monoterpene compounds present in the leaf extract of *E. pellita* had the ability to decrease chlorophyll content in leaves. This reduction is believed to be caused by the inhibition of chlorophyll producing and/or chlorophyll breakdown. In their study, Abd-Elgawad et al. (2020) identified α-pinene, β-pinene, 1.8-cineole, linalool, and carvacrol as the primary monoterpene chemicals in *E. pellita* that exhibit profound allelochemical effect activity. The allelochemical mechanism of plants, as described by Mondal et al. (2018), involves protein synthesis and the inhibition of microtubule formation. This inhibition is achieved by inhibiting tubulin polymerization, which is a crucial protein involved in the construction of cell walls. Consequently, the process of cell division ceases, so impacting the growth and development of plants.

According to Zakiyah et al. (2018), augmenting the chlorophyll content in plants enhances their capacity to harness sunlight and expedites the process of photosynthesis. Consequently, any disruption in the chlorophyll content leads to a reduced rate of photosynthesis. Photosynthesis, as described by Jhonson (2016), is the process of harnessing sunlight, water, and CO_2 to generate chemical energy required for the synthesis of organic matter and biomass. Disruptions to chlorophyll will also directly impede the process of photosynthesis.

Conclusion

The results of this work demonstrate that ethanol is a more effective solvent than aquadest for extracting Eucalyptus pellita leaves, resulting in a greater concentration of the 1.8-cineole molecule. The 1.8-cineole chemical obtained from the leaf extract of Eucalyptus pellita exhibits the greatest phytotoxicity against Borreria alata weeds when present at a concentration of 75%. However, its effectiveness is not significantly different when employed at a concentration of 50% compared to aquadest solvent.

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