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Detection of Terpenoid and Flavonoid of Five Species of Mistletoes at *Stelechocarpus burahol* (Bl.) Hook.f. & Th and *Lagerstroemia speciosa* (L.) Pers. by using Thin Layer Chromatography Method

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ABSTRACT
Mistletoe is a parasitic plant that is well known for causing significant damage to forestry plants; nevertheless, its value in traditional medicine has yet to be proven. This study aims to investigate terpenoid and flavonoid compounds of *Macrosolen cochinchinensis* (Lour.) Tiegh, *Scurrula atropurpurea* (Bl.) Dans., *Scurrula ferruginea* (Jack) Dans., *Dendrophthoe pentandra* (L.) Miq., *Dendrophthoe falcata* (L.f.) Ettingsh. found on *Stelechocarpus burahol* (Bl.) Hook.f. & Th, known as *Kepel*, and *Lagerstroemia speciosa* (L.) Pers, known as *Bungur*, using Thin Layer Chromatography (TLC). The research was conducted at Universitas Gadjah Mada's Department of Pharmaceutical Biology. Each mistletoe was extracted using dichloromethane for terpenoid detection, and ethanol 70% for flavonoid detection. Terpenoid and flavonoid compounds were detected using Thin Layer Chromatography (TLC) in the stationary phase of silica gel 60 F254. The mobile phase of n-hexane: ethyl acetate (93:7) was used for terpenoid detection, meanwhile both mobile phase of n-butanol: ethyl acetate: formic acid: water (5:5:2:1) and ethyl acetate: formic acid: water (90:5:5) were used for flavonoid detection. The result was further investigated under visible light and UV light 254 & 366 nm followed by anisaldehyde-H2SO4 spray reagent and thymol standard for terpenoid detection, and sitroborat spray reagent with rutin, quercetin, and quercitrin standard for flavonoid detection. Terpenoid was found on *hRf* 10, 20, 50, 60, 70, and 85 whereas it was found on *hRf* 30 for thymol standard. Flavonoid was found on *hRf*: 40, 50, 60, 70, and 85 whereas it was found on *hRf* 60 for rutin standard. Quercetin was found in mistletoe and its host on *hRf*: 60, however, quercitrin was only found in mistletoe species on *hRf* : 65.

Keywords: *Kepel, Bungur, Mistletoe, Thin Layer Chromatography, Terpenoid, Flavonoid, Quercitrin*

1. INTRODUCTION

Kepel (*Stelechocarpus burahol* (Bl.) Hook. f.) and *Bungur* (*Lagerstroemia speciosa* (L.)) are native plants of Southeast Asia commonly used for ornamental trees because of its beautiful appearance. *Kepel* is a cauliflorous plant of Annonaceae family which is commonly found in Java Province. It has been considered as an iconic plant and symbol of Daerah Istimewa Yogyakarta [1]. It is widely used as body odor repellant for women [2] *Kepel* contains the highest antioxidant in its fruits and there are most likely six chemical compounds discovered in its extract which plays an important role in antioxidant activity [3]. Antioxidant activity can also be found in its leaves [4] especially in the mature leaves which contain higher level of flavonoid than the younger leaves [5].

Meanwhile, *Bungur* is commonly known as ornamental tree because of its beautiful flower. Besides, *Bungur* can also be used in the degraded natural forest as a native restoration plant [6] because it is tolerant to degraded or critical land such as post-mining land [7]. *Bungur* also contains beneficial chemical compounds

such as carosolic acid found in its leaves extract that and commonly used for treating diabetes [8].

Despite its potential benefit in medication, *Kepel* and *Bungur* were known as potential host for mistletoes, the parasitic plants that invade their host by taking up water and nutrient using haustorium. There are several mistletoes genus widely distributed across Java viz. *Dendrophthoe*, *Scurrula*, *Lepeostegeres*, *Macrosolen*, and *Viscum* [9]. Mistletoe can invade various plant habitus such as shrubs and trees also and has been known to cause severe serious damage to forestry plants ranging from seedling to mature plants. The ecological and geographical conditions, host morphological characteristics, and abundance of dispersal agents (birds, insects, and mammals) are various factors that can contribute to mistletoe distribution. *Kepel* possess conical crown indicating larger gaps can be formed in the forest stand and it allows light intensity to increase as well as the risk of mistletoe infestation [10]. Meanwhile, *Bungur* has a thin trunk and can be damaged easily [11], therefore, mistletoe can potentially damage the tree.

Despite being a crucial problem for forestry plants, mistletoe benefit for traditional medication still needs to be observed substantially. *Dendrophthoe pentandra* found in *Kepel* exhibits antioxidant activity with IC50 value of (12.57 ±0.7) µg/mL and total phenolic content (13.76±0.9) mg gallic acid equivalents per gram of ethyl acetate fraction from ethanol extract of its leaves extract [12]. This study is aimed to observe beneficial terpenoid and flavonoid compounds from five mistletoes found in *Kepel* and *Bungur* particularly in the surrounding area of Universitas Gadjah Mada and identify their potential use for medication. Terpenoid and flavonoid compounds are phytochemical compounds derived from plants and have various benefits to improve human health. Terpenoid and flavonoidare phytochemical compounds mostly derived from plants and possess various benefits to improve human health. Thin Layer Chromatography (TLC) was used to detect terpenoid and flavonoid chemicals. Flavonoid was recognized using the rutin, quercetin, and quercitrin standards, whereas terpenoid was identified using the thymol standard.

2. MATERIALS AND METHODS

2.1. Plants Materials

Based on the Flora of Java [13], mistletoe was identified at the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada. *Kepel* and *Bungur* leaves along with the whole plant of five mistletoes (*Macrosolen cochinchinensis* (Lour.) Tiegh, *Scurrula atropurpurea* (Bl.) Dans., *Scurrula ferruginea* (Jack) Dans., *Dendrophthoe pentandra* (L.) Miq., and *Dendrophthoe falcata* (L.f.) Ettingsh) were collected in the surrounding area of Universitas Gadjah Mada. *Macrosolen cochinchinensis* (Lour.) Tiegh and *Scurrula atropurpurea* (Bl.) Dans. were collected from *Kepel.* Meanwhile, *Scurrula ferruginea* (Jack) Dans., *Dendrophthoe pentandra* (L.) Miq., and *Dendrophthoe falcata* (L.f.) Ettingsh., were collected from *Bungur*. The hosts of collected samples have passed the generative growth phase and possess a similar Diameter at Breast Height (DBH) to minimize the variability.

2.2. Chemical and Reagents

Materials for chemical constituent analysis are comprised of dichloromethane, ethanol, silica gel 60 F254 as stationary phase and n-hexane, ethyl acetate, n-butanol & formic acid as mobile phase. In this study we used two spray reagents namely anisaldehyde-H_2SO_4 and sitroborat along with thymol, rutin quercetin, and quercitrin as standards.

2.3 Thin Layer Chromatography

Kepel and *Bungur* leaves together with the whole plant of five mistletoes were dried using thermostat at 50^o C for 12 hours and ground until it became powder. For terpenoid detection, 3 grams of plant extract were extracted using 30 ml dichloromethane in an Erlenmeyer flask, followed by 2 hours of sonication. In addition, 3 grams of plant extract were utilized to detect flavonoids in 30 ml ethanol 70 percent. In the stationary phase of silica gel 60 F254, terpenoid and flavonoid components were identified using Thin Layer Chromatography (TLC). Terpenoid detection was carried out using the mobile phase of n-hexane: ethyl acetate (93:7), whereas flavonoid detection was carried out using the mobile phases of n-butanol: ethyl acetate: formic acid: water (5:5:2:1) and ethyl acetate: formic acid: water (90:5:5).

The result was further investigated under visible light and UV light 254 & 366 nm followed by anisaldehyde-H_2SO_4 spray reagent and thymol standard for terpenoid detection, and sitroborat spray reagent with rutin, quercetin, and quercitrin standard for flavonoid detection.

3. RESULTS AND DISCUSSIONS

There were five mistletoes of Loranthaceae family found on *Kepel* and *Bungur* in the surrounding area of Universitas Gadjah Mada (Figure 1) viz. *M. cochinchinensis*, *S. atropurpurea*, *S. ferruginea*, *D. pentandra*, and *D. falcata*. Mistletoe has no particular host specificity, therefore, each species can invade a wide range of plants. For instance, *D. pentandra* can invade both *S. burahol* and *Lagerstroemia* sp. [14].

Figure 1 Mistletoes found at *Kepel* and Bungur in the surrounding area of Universitas Gadjah Mada**.** (A: (*M. cochinchinensis*) and B: (*S. atropurpurea*) in *Kepe*l; C: (*S. ferruginea*) D: (*D. pentandra*) and E: (*D. falcata*) in *Bungur*)

Each mistletoe has particular morphological traits. *Macrosolen* (Genus *Macrosolen*) is a shrub with dense branches and increasing internodes on its branches (Figure 1A). The leaves of the *Macrosolen* genus have a short petiole which is elliptical, lanceolate, or ovate in shape, and the leaves are as thick as human skin. The leaves, on the other hand, appear to be slick on all sides. Meanwhile, Genus *Scurrula* (Figure 1B and Figure 1C) has simple obovate leaves with an opposite leaf arrangement. It has elliptical or obovate shape. The length and the width ranged from 5-9 cm and 2-4 cm respectively. Also, the tawny trichomes cover the lower side of the leaf surface (abaxial).

Genus *Dendophthoe* (Figure 1D and Figure 1E) is also classified as shrub and it can reach more than 1 m in height. Dendrophthoe leaves are alternately arranged, have a short petiole, and are lanceolate to ovate or oblong in shape, with length and width ranging from 5-20 cm and 2-12 cm, respectively. Furthermore, after

being picked from the main stem, its leaves might easily discharge [13].

Terpenoid and flavonoid were found in all mistletoe species in *Kepel* and *Bungur*, according to the findings. Terpenoid and flavonoid are phytochemical compounds that are found in varied concentrations in plants, microorganisms, animals, and other forms of life, and coexist in the metabolic system alongside other bioactive compounds such as alkaloids, polyphenols, and steroid.[15].

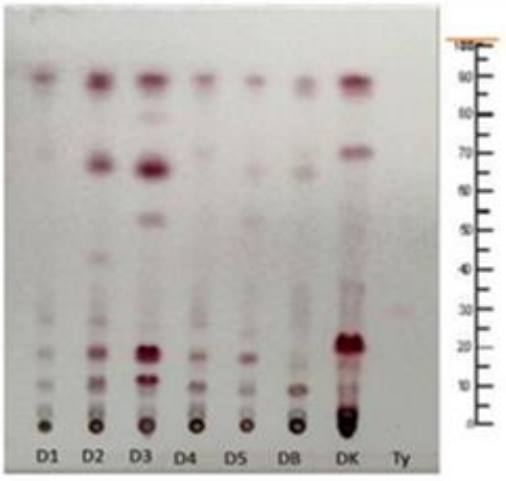

Figure 2 Chromatogram pattern of Dichloromethane extract of Kepel, Bungur, and their mistletoes for detection of terpenoid with thymol standard**.** (D: Dichloromethane; 1: *M. cochinchinensis*, 2: *S. atropurpurea*, 3: *S. ferruginea*, 4: *D. pentandra*, 5: *D. falcata*, B: *Bungur*, K: *Kepel*)

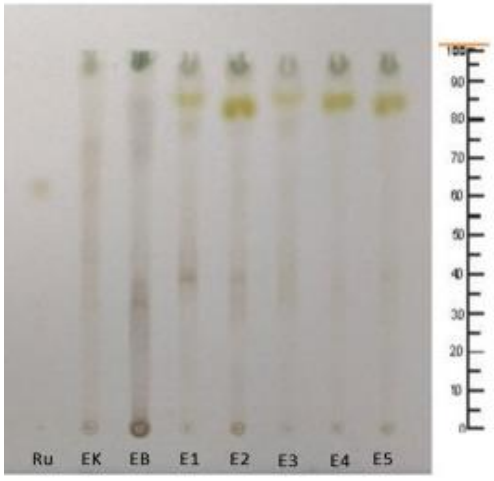

Figure 3 Chromatogram pattern of ethanol 70% extract of *Kepel, Bungur,* and their mistletoes for detection of flavonoid with rutin standard. (E: Ethanol; 1: *M. cochinchinensis*, 2: *S. atropurpurea*, 3: *S. ferruginea*, 4: *D. pentandra*, 5: *D. falcata*, B: *Bungur*, K: *Kepel)*

Terpenoid compound is indicated by rosy or purplish spots resembling the color of thymol standard after being sprayed with anisaldehyde-H2SO4 and heated at 110 ^{o}C [15]. Terpenoid was found in mistletoe and its host (Figure 2). According to chromatogram, terpenoid of mistletoe and its host was found on *hRf* 10, 20, 50, 60, 70, and 85 whereas terpenoid of thymol standard was found on *hRf* 30.

All mistletoe species and their hosts contained flavonoid chemicals (Figure 3). After being sprayed with sitroborat, they produce yellowish or tawny patches that resemble the rutin standard reaction. [16]. Flavonoids are a diverse group of around 4000 secondary plant metabolites that are usually found as sugar conjugates. [17]. According to chromatogram, flavonoid was found on *hRf*: 40, 50, 60, 70, and 85 whereas flavonoid of rutin standard was found on *hRf* 60. Unfortunately, the optimization of mobile phase using n-hexane: ethyl acetate (93:7) and n-butanol: ethyl acetate: formic acid: water (5:5:2:1) was still low because the compounds were not completely separated.

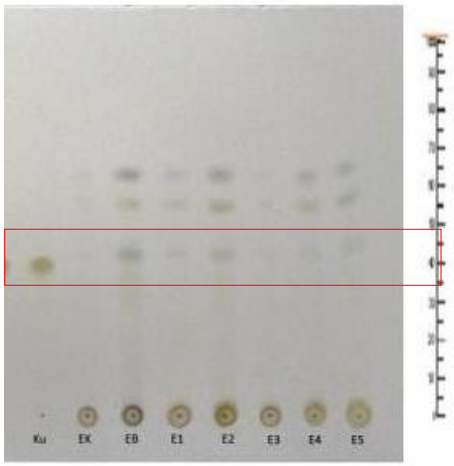

Figure 4 Chromatogram pattern of ethanol 70% extract of *Kepel, Bungur,* and their mistletoes for flavonoid detection using quercetin standard. (E: Ethanol; 1: *M. cochinchinensis*, 2: *S. atropurpurea*, 3: *S. ferruginea*, 4: *D. pentandra*, 5: *D. falcata*, B: *Bungur*, K: *Kepel*)

Another flavonoid compound found in the present study was quercetin (Figure 4). The chromatogram showed that all mistletoes and their host contain quercetin on *hRf* 60. Quercetin mostly found in vegetables and it also can be found in woody plants such as guava (*Psidium guajava)*, cashew (*Anacardium occidentale*), or hummingbird tree (*Sesbania grandiflora*) [17]. The benefit of quercetin as medication has been widely acknowledged. It possesses several beneficial biological activities, for instance, antioxidant, anti-inflammatory, anti-cancer, and anti-viral properties [18].

On the other hand, flavonoid detection using mobile phase of ethyl acetate: formic acid: water (90:5:5) successfully obtained quercitrin which was only found in the mistletoe species (Figure 5). Quercitrin is also indicated by yellowish or tawny spots. Quercitrin was found in all mistletoe species on *hRf* : 65. Quercetin (4H-1-benzopyran-4-0n2, 2-(3,4-dihydroxyphenil)-3,5,7-trihydroxy-flavon) and quercitrin (2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl6-deoxyalpha-L mannopyranoside) are biological flavonoids with slightly different structure in its benzene rings and found in a variety of plants' flowers, leaves, and fruits [19]. The slightly different position indicates distinct anti-inflammatory and antioxidation activities, which could be a future study focus, particularly for mistletoe-derived quercetin or quercitrin.

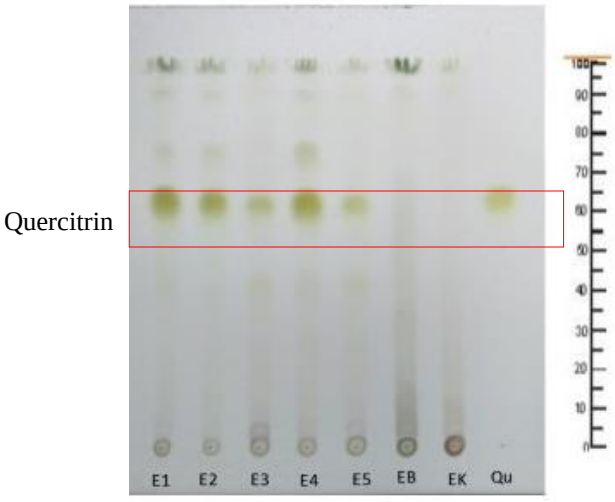

Figure 5 Chromatogram pattern of ethanol 70% extract of *Kepel*, *Bungur*, and their mistletoes.for flavonoid detection using quercitrin standard**.** (E: Ethanol; 1: *M. cochinchinensis*, 2: *S. atropurpurea*, 3: *S. ferruginea*, 4: *D. pentandra*, 5: *D. falcata*, B: *Bungur*, K: *Kepel*)

It is suspected that flavonoid compound in the form of quercetin was translocated into the mistletoes by its host and being converted into quercitrin. This compound is considered beneficial to treat individuals suffering from hepatitis, cirrhosis or hepatic cancers [20]. Therefore, it needs further observation to examine the clinical trials of quercitrin found in mistletoes of *Kepel* and *Bungur* as traditional medication.

AUTHORS' CONTRIBUTIONS

All authors contributed to the study conception and design. DS performed the experiments, analysed the data, and provided the draft for the manuscript. SMW provided an insightful concept and valuable feedback for the manuscript. UR and BS composed the English manuscript and provided supporting references. All authors read and approved the final manuscript.

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