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Phytochemical and Thin Layer Chromatography Analyses of *Lophopetalum wallichii* Kurz Barks Native to Cambodia

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ABSTRACT

Lophopetalum wallichii Kurz native to Cambodia has been widely used for healing various ailments such as asthma, urinary disorders, influenza, arthritis, malaria, and cancers. This study aimed at identifying the phytochemical components of ethanolic extracts of the *Lophopetalum wallichii* Kurz barks, native to Cambodia, and at profiling its Thin Layer Chromatography (TLC) fingerprints. The dried barks of *Lophopetalum wallichii* Kurz were subjected to the Ultrasound-Assisted Extraction (UAE) with ethanol; the extracts were subsequently preserved for the analyses of phytochemicals and TLC. The phytochemical screening of *Lophopetalum wallichii* Kurz revealed positive tests of alkaloids, phenolic compounds, tannins, flavonoids, terpenoids, cardiac glycosides, saponins and resins. TLC was developed with a mobile phase system Chloroform:Ethanol (1:1) and investigated under 254-366 nm UV light and 10%-H_2SO_4, and provided a clear separation with different R_f values. These findings together could be used for the isolation of bioactive pure compounds.

Keywords: Lophopetalum wallichii; Thin layer chromatography

INTRODUCTION

Lophopetalum spp. is represented by about 18 species of evergreen trees that grow in Cambodia, India, Laos, Malaysia, Myanmar, Thailand, and Vietnam (Sturm et al., 1996). *Lophopetalum wallichii* Kurz native to Cambodia is locally called "*Puen Ta Lei*" (Leti et al., 2013) and is under the family Celastraceae traditionally being used to treat various disorders such as asthma, urinary infections, malaria, influenza, fever, arthritis, rheumatism, sprains, mental diseases, tuberculosis, body pain, cancers, menstrual diseases, dysmenorrhea, menorrhagia, stomach upset, ulcers, skin eruptions, syphilis, gonorrhea, leprosy, respiratory infections, and autoimmune diseases (González et al., 2000). Phytochemicals or secondary metabolites are referred to as non-nutrient plant chemical compounds

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or bioactive components playing an important role in protecting the plant against various diseases and herbivores (Doughari, 2012). Parts of plant such as roots, stems, leaves, flowers, fruits or seeds are accumulated with phytochemical components which are classified into different drug groups including alkaloids, flavonoids, glycosides, tannins, saponins, phenolics, and terpenoids (Saxena, 2013). These constituents potentially possess such illness-curative effects as rheumatoid arthritis, haemorrhagic shock, cardiovascular diseases, cystic fibrosis, metabolic disorders, neurodegenerative diseases, and gastrointestinal ulcer oogenesis (Patel et al., 2015). Thin-layer chromatography (TLC) is a technique which is used to separate mixtures containing in medicinal plants. TLC can be executed on a sheet of glass, plastic, or aluminum foil, which is covered with a thin layer of adsorbent material, usually silica gel, cellulose or aluminum oxide (Preethi, 2017). The solvent applied for the separation is used as the "*mobile phase*" whereas the absorbent materials are used as the "*stationary phase*," the both phases are different in polar properties (Ahamed et al., 2017). TLC is used as a "fingerprint" method for the characterization of a plant extract. The samples and the reference standards are applied on the same plate. After the elution with a suitable solvent mixture, the plate is examined in UV light 254 or 366 nm, with or without derivatization. A simple identification is comparing the R_f values of a separated spot in the sample and of the reference compounds (Gocan & Cimpan, 2004). Here we report TLC profiles of the barks of *Lophopetalum wallichii* Kurz native to Cambodia. This biodata is of importance for its validation and standardization; therefore, owing to the scant attention to the phytochemicals in this Cambodian *Lophopetalum wallichii* Kurz.

MATERIAL AND METHODS

Collection of plants: The dried barks of *Lophopetalum wallichii* Kurz were collected from the local drugstore selling medicinal plants in Phnom Penh, Cambodia, in April 2017. The plant was authenticated with the voucher specimen (UPFPT-110066) of University of Puthisastra (UP)-Herbarium (Figure 1). A part of the plant sample was deposited in the UP-Herbarium and the Pharmacognosy Laboratory, Department of Pharmacy (DoP), Faculty of Health Sciences (FHS), University of Puthisastra (UP), with a purpose of further conducting investigation. The dried barks of *Lophopetalum wallichii* Kurz were further curtailed and extracted with ethanol in Ultrasonicator Elmasonic S100H 50/60 Hz, Germany. The broth was filtered and evaporated at room temperature in a fume hood. The filtrate was concentrated to obtain the crude extract; the extract was in turn subjected to the phytochemical and TLC analyses. The dried extract was properly stored in the desiccators for further experiments and analyses.

Phytochemical analysis: The ethanolic extract of *Lophopetalum wallichii* Kurz barks underwent the phytochemical screening in order to detect the presence (or the absence) of alkaloids, tannins, flavonoids, coumarins, cardiac glycosides, phenolics, resins, saponins, terpenoids, polypeptides and essential oils by using the standard methods (Harborne, 1984).

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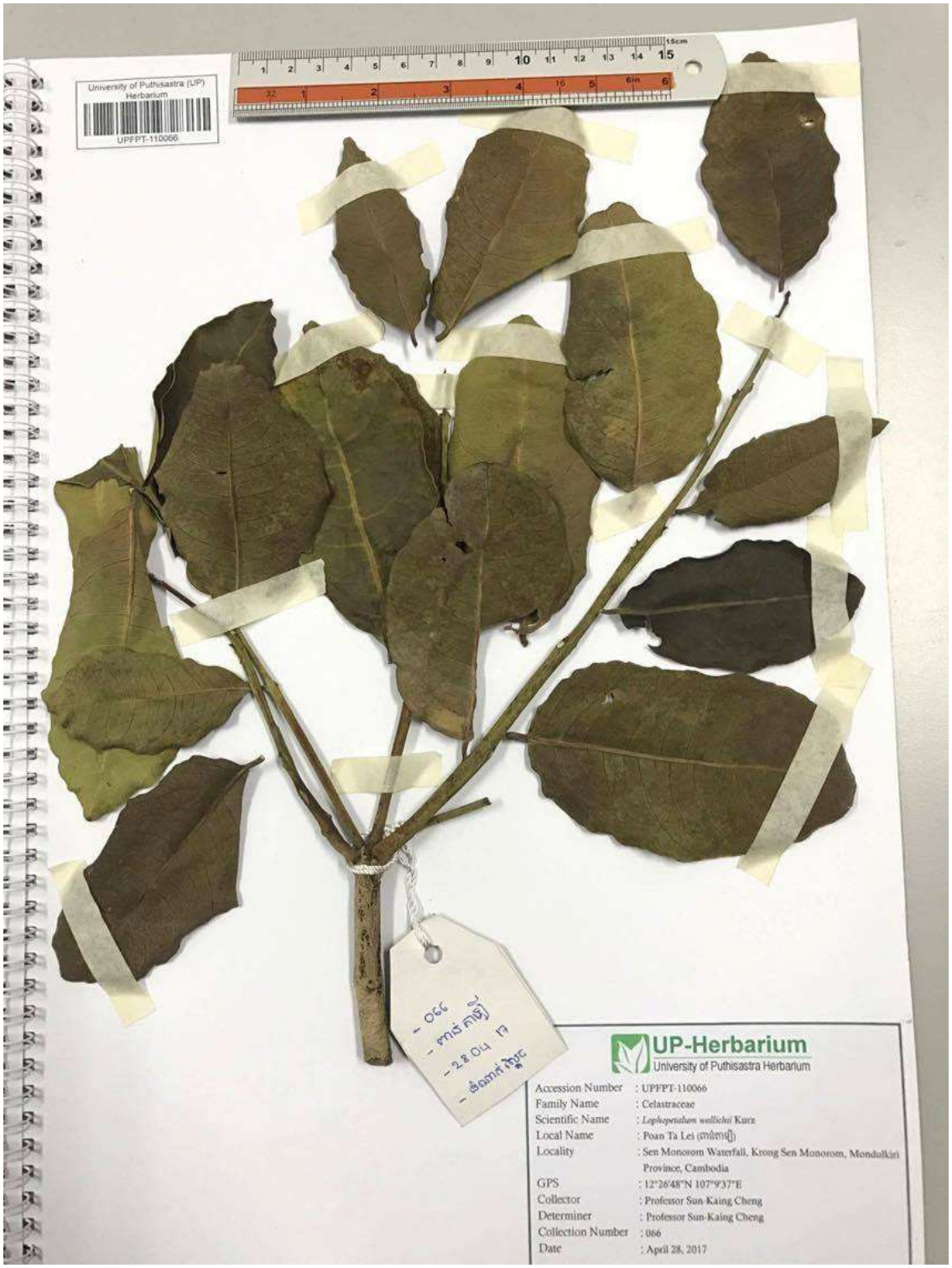

Figure 1. Voucher specimen of *Lophopetalum wallichii* Kurz deposited in UP-Herbarium.

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Test for alkaloids (Dragendorff's, Mayer's and Wagner's tests): Eight-milliliter filtrate of the extract was loaded equally into four test tubes. One control test tube was added with no reagent, and the rest of test tubes were treated with Dragendorff's, Mayer's or Wagner's reagents. The orange red (Dragendorff), creamy white (Mayer) or reddish brown (Wagner) precipitates demonstrated the presence of Alkaloids (Humayun et al., 2012).

Test for phenolic compounds (Ferric Chloride test): Two-milliliter filtrate of the extract was pipetted into the test tube, following with the addition of distilled water 5 ml. Four drops of 5%-$FeCl_3$ were dripped into the filtrate. The formation of dark green precipitate indicated the presence of Phenolic Compounds (Raaman, 2006).

Test for tannins (Ferric Chloride test): The extract of two-milliliter filtrate was transferred into the test tube and added with a few drop of 0.1%-$FeCl_3$. The tested group was compared with the control group, which was not added with the reagent. The blue-black coloration interpreted the presence of Tannins (Karthishwaran et al., 2010).

Test for flavonoids (Ammonium test): The extract was dissolved in chloroform 2 ml and taken into the test tube. One milliliter of 1%-NH_4 was added into it. The mixture was shaken vigorously. The information of yellow color observed in the ammonia layer demonstrated the presence of Flavonoids (Sheel et al., 2014).

Test for coumarins (NaOH test): Two-milliliter of extract filtrate was loaded into the test tube and added with 3 ml of 10%-NaOH. Basically, the yellow coloration represents of Coumarins (Sawant & Godghate, 2013); however, the coloration of yellow was not observed in this test.

Test for steroids (Liebermann–Burchard test): The extract 100 mg was dissolved in the chloroform 2 ml and filtered into the test tube. The mixture was added with 1 ml of glacial acetic acid, followed by carefully the addition of 1 ml of H_2SO_4 along the side of the test tube. Fundamentally, the greenish color indicates the presence of Steroids (Bargah, 2015); nevertheless, this test showed no greenish color observation.

Test for terpenoids (Salkowski's test): The extract of 100 mg was dissolved in the chloroform 5 ml and filtered into the test tube. The mixture was added carefully with 3 ml of H_2SO_4 along the side of the test tube. The reddish brown color at the interface of the two phases characterized the presence of Terpenoids (Ajiboye et al., 2013).

Test for cardiac glycosides (Keller–Kiliani's test): The glacial acetic acid 2 ml was mixed with 2 drops of 2%-$FeCl_3$. The extract 100 mg was dissolved in this solution in the test tube. The mixture was added with 1 ml of H_2SO_4 along the side of the test tube. The brown ring at the interface indicated the presence of Cardenolides, and the violet-green ring below the brown ring in the acetic acid layer represented Glycoside. These together characterized Cardiac Glycosides (Ajiboye et al., 2013; Jaradat et al., 2015).

Test for essential oils (NaOH–HCl test): In a test tube, the filtrate 2 ml of the extract was added with 100 µl of 1M-NaOH, followed by the addition of 3 drops of 1M-HCl. The mixture was shaken.

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The white precipitate, basically, demonstrates the presence of Essential Oils (Mir et al., 2013); however, this test was none of the white precipitate observed.

Test for saponins (Froth test): The distilled water 15 ml were added to 100 mg of the extract and filtered into the test tube. The mixture was shaken for 10 min until the formation of stable persistent froth. Formation of stables five-minute persistent froth indicated the presence of Saponins (Djaafar & Ridha, 2014).

Test for resins (Turbidity test): Ten milliliter of distilled water were added to 200 mg of the extract and filtered into the test tube, and the mixture was observed. The occurrence of turbidity showed the presence of Resins (Mir et al., 2013).

Thin Layer Chromatography (TLC): The ethanolic extract was subjected to the TLC as per conventional one-dimensional ascending method using Silica gel 60 F254 (Merck) as stationary phase. The mobile phase ratio: Chloroform: Ethanol (1:1) was formulated. The CAMAG UV Lamp with 254-366 nm wavelengths and the 10%-H_2SO_4 reagent were used for the detection of compound spots onto the eluted TLC plate. The movement of the compounds was expressed by its retention factor (R_f), the values of which were calculated based on the following equation (Sarkar et al., 2011).

$$R_f = \frac{\text{Compound distance from origin}}{\text{Solvent front distance from origin}}$$

RESULTS

Phytochemical constituents: The barks of *Lophopetalum wallichii* Kurz showed the positive test of all phytoconstituents including alkaloids, phenolic compounds, tannins, flavonoids, terpenoids, cardiac glycosides, saponins and resins, except for coumarins, steroids and essential oils (Table 1).

Fingerprints by Thin Layer Chromatography: The TLC study of the ethanolic extract of *Lophopetalum wallichii* Kurz barks, under the mobile phase system of Chloroform:Ehtanol (1:1), indicated that 2 spots were visible of R_f values 0.07 and 0.96 detected by 254 nm UV; that 1 spots were visible of R_f values 0.97 detected by 366 nm UV; and that 7 spots were visible of R_f values 0.08, 0.24, 0.30, 0.34, 0.77, 0.90 and 0.96 detected by 10%-H_2SO_4 (Table 2; Figure 2).

DISCUSSION

This study was carried out on the barks of *Lophopetalum wallichii* Kurz which indicated that it contains the phytochemical components such as alkaloids, phenolic compounds, tannins, flavonoids, terpenoids, cardiac glycosides, saponins and resins, and the results are summarized in Table 1.

Table 1. Phytochemical screening of the ethanolic extract of *Lophopetalum wallichii* Kurz barks.

Phytochemical Constituents	Types of Chemical Tests	Ethanolic Extract of *Lophopetalum wallichii* Kurz
Alkaloids	Dragendorff's	Positive

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	Mayer's	Positive
	Wagner's	Positive
Phenolic compounds	Ferric chloride	Positive
Tannins	Ferric chloride	Positive
Flavonoids	Ammonium	Positive
Coumarins	NaOH	Negative
Steroids	Liebermann–Burchard	Negative
Terpenoids	Salkowski's	Positive
Cardiac glycosides	Keller–Kiliani's	Positive
Essential oils	NaOH–HCl	Negative
Saponins	Froth	Positive
Resins	Turbidity	Positive

These phytochemicals possess the biological mechanism of action including antioxidant effects, modulation of enzyme actions, stimulation of the immune system, modulation of hormone metabolism, antibacterial and antiviral effects, and interference with DNA replication (Sospeter et al., 2013), thereby leading to the treatment of various diseases such as pneumonia, kidney ailments, atherosclerosis, rheumatism, asthma, gastrointestinal disorders, hepatitis, tuberculosis, cancers, and allergy (Egamberdieva, (2017). In accordance with our study, flavonoids, phenols and tannic acids were positively tested in the *Lophopetalum* sp (Ansari et al., 2015). Balayer et al. (1993) isolated two types of alkaloids bhesine and dehydrobhesine, which was structurally elucidated by NMR and X-Ray single crystal analysis, in the plant belonging to the family Celastraceae. Similarly, in this plant family, Callies et al. (2017) also reported the isolation of some sesquiterpene pyridine alkaloids such as vulcanicoline-A, cuzcoinine, vulcanicoline-B, jelskiine, and vulcanicoline-C under the method of NMR spectroscopic analysis. These are in agreement with our study of the alkaloid test indicating the

Table 2. R_f values of the ethanolic extract of barks of *Lophopetalum wallichii* Kurz detected by 254-366 nm UV and 10%-H_2SO_4. MPS = Mobile Phase System.

Medicinal Plant	Detectors	R_f values [MPS: Chloroform:Ethanol (1:1)]
Barks of *Lophopetalum wallichii* Kurz	254 nm UV	0.07, 0.96
	366 nm UV	0.97
	10%-H_2SO_4	0.08, 0.24, 0.30, 0.34, 0.77, 0.90, 0.96

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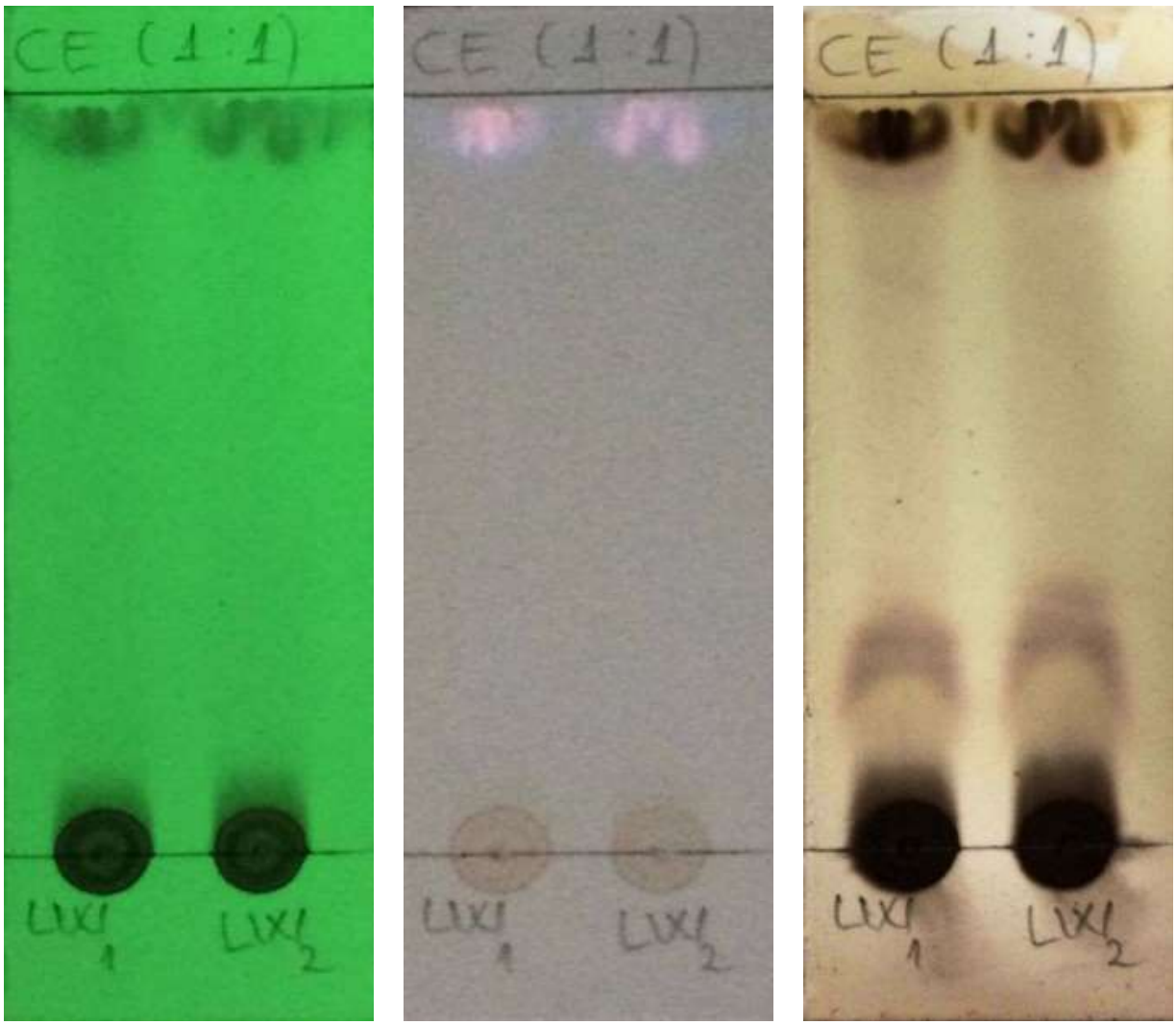

254 nm UV 366 nm UV 10%-H_2SO_4

Figure 2. TLC analysis of ethanolic extract *Lophopetalum wallichii* Kurz barks under the mobile phase system Chloroform:Ethanol (1:1).

presence of the alkaloids in *Lophopetalum wallichii* Kurz. Alkaloids isolated from the Celastraceae plants showed inhibitory effects on the proliferation of human rheumatoid arthritis synovial fibroblast cell (MH7A) at a concentration of 20 μM Fan et al., (2016). Moreover, the lupane-type triterpenes including ochraceolide A, ochraceolide B, betulin, lupeol, and dihydroochraceolide A isolated from the *Lophopetalum wallichii* Kurz exert the Farnesyl Protein Transferase (FPT) inhibitory effect (Sturm et al., 1996). Cardiac glycosides could be also found in some plants, especially, the *Lophopetalum* sp. which is found to have the therapeutic effect of poisoning and congestive heart failure (Morsy, 2017), which is in agreement with the positive test for cardiac glycosides in our observation. Moreover, we also found the presence of the saponins. Saponins in the family Celastraceae have been found to exert antidiarrheal activity via antimotility and antisecretory mechanisms (de Oliveira et al., 2017). The positive test for resins we observed in our study is consistent with Mercadante-Simões & Paiva (Mercadante-Simões & Paiva, 2013). Plant resins inhibit the infections caused by bacterial strains

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(Shuaib et al. 2013). The TLC profiling of the ethanolic extract of *Lophopetalum wallichii* Kurz barks gave good separation of the phytochemicals with different R_f values reflecting an idea about their polarity. Keeragalaarachchi et al. (2016) reported a similar finding of TLC fingerprints expressing different R_f values of the methanolic extracts of Celastraceae species observed under the UV detection of 366 nm and after spraying vanillin sulfuric; the mobile phase system was Cyclohexane:Dichloromethane:Ethyl acetate:Methanol (3:1:0.3:0.4). Several reports fingerprinted the medicinal plants onto the TLCs expressing different R_f values of various compounds providing valuable clues regarding their polarity and selection of solvents for the separation of phytochemicals (Banu & Nagarajan 2014; Karthika & Paulsamy, 2015).

CONCLUSION

This study provides evidence on the phytochemical components including alkaloids, phenolic compounds, tannins, flavonoids, terpenoids, cardiac glycosides, saponins and resins in the ethanolic extract of *Lophopetalum wallichii* Kurz barks. This plant's TLC layouts of the spots detected with 254-366 nm UV light and 10%-H_2SO_4 reagent give good separation with the mobile phase system of Chloroform:Ethanol (1:1). It can be used for authentication and the standardization for the quality control of *Lophopetalum wallichii* Kurz and contribute to future research in term of natural drug isolation.

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DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

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