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Research Article

Phytochemical and Thin Layer Chromatography Analyses of *Dillenia ovata* Wall. ex Hook.f. & Thomson Barks Native to Cambodia

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Abstract

Dillenia ovata Wall. ex Hook.f. & Thomson species are broadly used by the traditional healers in Southeast Asia as they are of benefit for the treatment of various diseases such as hemorrhoids, diarrhea, dysentery, and wound healing. This study was conducted to identify the phytochemical constituents of the ethanolic extracts of the barks of *Dillenia ovata* Wall. ex Hook.f. & Thomson and to record its profile of Thin Layer Chromatography (TLC). The barks of *Dillenia ovata* Wall. ex Hook.f. & Thomson were subjected to the Ultrasound-Assisted Extraction (UAE) with ethanol; the yielded ethanolic extracts were, in turn, taken to phytochemical and TLC analyses. The phytochemical analysis of the ethanolic extracts of *Dillenia ovata* Wall. ex Hook.f. & Thomson showed that the barks of *Dillenia ovata* Wall. ex Hook.f. & Thomson contained alkaloids, tannins, phenolic compounds, coumarins, cardiac glycosides, resins, saponins, flavonoids, polypeptides, and terpenoids. The TLC analysis, evaluated with the mobile phase system of Chloroform:Ethanol (15:1) and investigated under 254-366 nm UV light and 10%-H_2SO_4, provided a clear separation with different R_f values. Collectively, it is concluded that the presence of these phytochemicals and the TLC profiling of the *Dillenia ovata* Wall. ex Hook.f. & Thomson barks may be responsible for its medicinal purpose and be of benefit for future research in term of natural drug isolation.

Key words: *Dillenia ovata*, UAE, phytochemical, TLC, R_f value

Introduction

Cambodia, located in Southeast Asia, is rich of natural resources and biodiversity and considered largely the forested as the vascular plants alone of over 3000 species have been identified (Chassagne et al., 2016). Medicinal plants are useful for healing as well as for curing of human diseases because of the presence of their phytochemical constituents. Moreover, their therapeutic activities are generally in their different parts such as roots, leaves, fruits, and barks (Bargah, 2015).

The Cambodian *Dillenia ovata* Wall. ex Hook.f. & Thomson (Khmer name: Phlu Thôm) (family Dilleniaceae) has been traditionally used as a medicinal plant for centuries, and it remains practiced until today (Dy, 2000). The *Dillenia* spp. play a critical role in traditional medicine, and they have been used for the treatment of various diseases and infections, such as arthritis, diabetes,

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dysentery, hepatitis, blennorrhagia, and to treat gastrointestinal disorders, inflammation, hemorrhoids, wounds, and leishmanial ulcers. The pharmacological studies have confirmed that extracts from these species as well as some of their isolated compounds possess a wide range of biological activities including anti-hemorrhagic, anti-inflammatory, antioxidant, antimicrobial, antitumoral, anti-ulcer, immunological, and cancer chemoprevention (Lima et al., 2014).

Phytochemicals, comprised of primary and secondary compounds, are naturally occurring in the medicinal plant or its parts including leaves, barks, fruits, and roots which possess defense mechanisms and prevent from different illnesses (Wadood et al., 2013). The most important bioactive phytochemicals of plants are alkaloids, tannins, flavonoids, cardiac glycosides and phenolic compounds. The correlation between the phytocomponents and the bioactivity of plants are also desirable to know with specific activities to cure various health ailments and a number of chronic diseases (Yadav et al., 2014).

Thin layer chromatography (TLC) is the most broadly used method for the characterization of plant extracts, essential oils, medicinal plants, and other products derived from plants. Basically, the TLC is used as a "fingerprint" method for the characterization of plant extracts. The samples and the references are applied on the same plate. After the elution with a suitable solvent mixture, the plate is examined in Vis or UV light (254 or 366 nm), with or without derivatization. A simple identification is comparing the R_f values of the separated spots in the sample and of the reference standard (Gocan & Cimpan, 2004).

This study points out the identification of phytochemical components and the TLC profiles available in the barks of *Dillenia ovata* Wall. ex Hook.f. & Thomson native to Cambodia. This information is of importance for the validation and the standardization of the Cambodian medicinal plants; therefore, because of the scant attention to the phytochemicals in this Cambodian *Dillenia ovata* Wall. ex Hook.f. & Thomson, we decided to conduct an observation. This aimed to report the phytochemical constituents and TLC profile embedded in the dried barks of *Dillenia ovata* Wall. ex Hook.f. & Thomson native to Cambodia.

Materials and methods

Collection of plants

The dried barks of *Dillenia ovata* Wall. ex Hook.f. & Thomson 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-110057) 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), on a purpose of conducting further investigation.

Preparation of plant extracts

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Figure 1. Voucher specimen of *Dillenia ovata* Wall. ex Hook.f. & Thomson in UP-Herabrium

The dried barks of *Dillenia ovata* Wall. ex Hook.f. & Thomson 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 evaluation and the TLC analysis. The dried extract was properly stored in the desiccators for further experiments and analyses.

Phytochemical analysis

The ethanolic extract of barks of *Dillenia ovata* Wall. ex Hook.f. & Thomson underwent the phytochemical screening in order to detect the presence (or the absence) of alkaloids, tannins,

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flavonoids, coumarins, cardiac glycosides, phenolics, resins, saponins, terpenoids, polypeptides and essential oils by using standard methods (Harborne, 1984).

Test for alkaloids (Dragendorff's, Mayer's and Wagner's Tests): 15 mL filtrate of the extract was loaded equally into four test tubes. One test tube was kept as the control group without adding any reagent into it. The remains were treated with few drops of Dragendorff's, Mayer's or Wagner's reagents. The orange red (Dragendorff), creamy white (Mayer) or reddish brown (Wagner) precipitates indicated the presence of alkaloids (Humayun et al., 2012).

Test for phenolic compounds (Ferric Chloride Test): 5 mL of the extract filtrate was transferred into the test tube and added with 3 drops of 5%-$FeCl_3$. The tested solution changed the color to dark green in comparison with the control group. This chemical reaction indicated the presence of Phenolic Compounds (Raaman, 2006).

Test for tannins (Ferric Chloride Test): 10 mL of the extract filtrate was transferred to the test tube and added with 5 drops of 0.1%-$FeCl_3$. The tested group was compared with the control group, which was not added with the reagent. The tested solution gave the brownish green coloration indicating the presence of Tannins (Karthishwaran et al., 2010).

Test for flavonoids (Ammonium Test): 10 mL of the extract filtrate was loaded into the test tube and added with 1ml of 1%-ammonium solution. The mixture was shaken vigorously. The observed yellow color at the ammonia layer indicated the presence of Flavonoids (Sheel et al., 2014).

Test for coumarins (NaOH Test): 10 mL of the extract filtrate were added with 3 drops of 10%-NaOH. The yellow color indicated the presence of Coumarins (Sawant & Godghate, 2013).

Test for terpenoids (Salkowski Test): 5 mL chloroform was added to the extract of 100 mg 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 liquids characterized the presence of Terpenoids (Ajiboye et al., 2013).

Test for cardiac glycosides (Killer-Killiani Test): 2 mL of glacial acetic acid with 3 drops of 2%-$FeCl_3$ were added to 100 mg of the extract 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 (Jaradat et al., 2015).

Test for essential oils (NaOH-HCl Test): 10 mL of the extract filtrate in the test tube was added with 3 drops of 1M-NaOH and added with 1M-HCl. The white precipitates confirm the presence of Essential Oils (Mir et al., 2013), while this test was not positive of Essential Oils (Mir et al., 2013).

Test for resins (Turbidity Test): 10 mL 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).

Test for saponins (Froth Test): 200 mg of the extract were added with 10 ml of distilled water in the test tube, and the mixture was sonicated for 5 min. Five milliliters of distilled water were added to the mixture and shaken vigorously for 10 min until the formation of stable persistent froth. The formation of one-centimeter layer stable persistent froth for 5 min indicated the presence of Saponins (Djaafar & Ridha, 2014).

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Test for polypeptides (Biuret Test): 3 mL of the extract filtrate were added with 1 ml of 40%-NaOH, followed by the addition of 2 drops of 1%-$CuSO_4$. The violet color indicated the presence of Polypeptides (Santhi & Sengottuvel, 2016).

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 the stationary phase. The mobile phase of ratio: Chloroform:Ethanol (15:1) 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 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):

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

Results

Phytochemical Constituents

The barks of *Dillenia ovata* Wall. ex Hook.f. & Thomson showed the positive test of all phytochemicals covering alkaloids, phenolic compounds, tannins, flavonoids, coumarins, cardiac glycosides, terpenoids, resins, saponins, and polypeptides, except for essential oils (Table 1).

Table 1: Phytochemical analysis of the ethanolic extract of *Dillenia ovata* Wall. ex Hook.f. & Thomson barks

Phytochemical Constituents	Chemical Tests	Ethanolic Extracts of *Dillenia ovata* Wall. ex Hook.f. & Thomson
Alkaloids	Dragendorff's test	Positive
	Mayer's test	Positive
	Wagner's test	Positive
Phenolic compounds	Ferric Chloride test	Positive
Tannins	Ferric Chloride test	Positive
Flavonoids	Ammonium test	Positive
Coumarins	NaOH test	Positive
Cardiac glycosides	Killer-Killiani test	Positive
Terpenoids	Salkowski test	Positive
Essential oils	NaOH-HCl test	Negative
Resins	Turbidity test	Positive
Saponins	Froth test	Positive
Polypeptides	Biuret test	Positive

Thin layer chromatography

The TLC study of the ethanolic extract of *Dillenia ovata* Wall. ex Hook.f. & Thomson barks, under the mobile phase system of Chloroform:Ehtanol (15:1), indicated that 5 spots were visible of R_f values 0.43, 0.57, 0.69, 0.80, and 0.86 detected by 254 nm UV; that 6 spots were visible of R_f values

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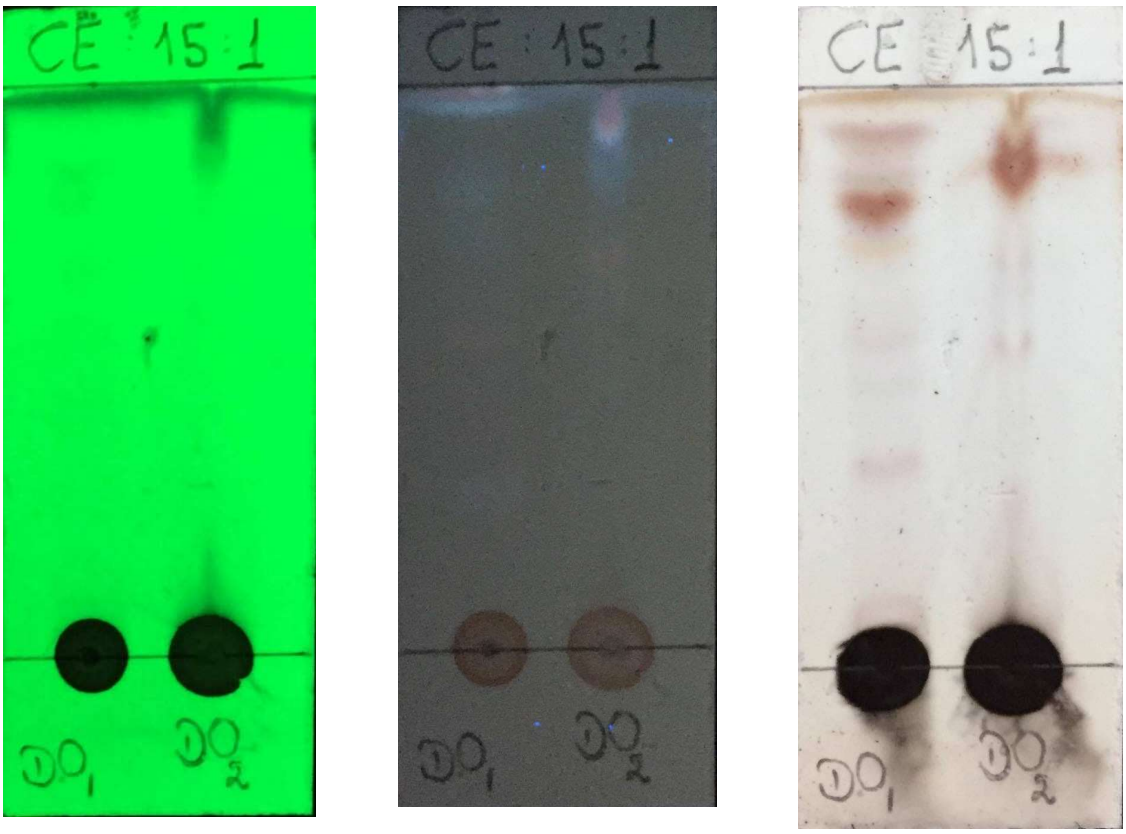
0.26, 0.49, 0.54, 0.74, 0.83, and 0.94 detected by 366 nm UV; and that 9 spots were visible of R_f values 0.11, 0.34, 0.49, 0.57, 0.63, 0.74, 0.80, 0.86, and 0.94 detected by 10%-H_2SO_4 (Figure 2; Table 2).



254 nm 366 nm 10%-H_2SO_4

Table 2: R_f values of the ethanolic extracts of *Dillenia ovata* Wall. ex Hook.f. & Thomson barks detected by 254-366 nm UV and 10%-H_2SO_4. MPS = Mobile Phase System.

Medicinal Plant	**Detectors**	**R_f values [MPS: Chloroform:Ethanol (15:1)]**
Ethanolic Extract of Barks of *Dillenia ovata* Wall. ex Hook.f. & Thomson	254 nm UV Lamp	0.43, 0.57, 0.69, 0.80, 0.86
	366 nm UV Lamp	0.26, 0.49, 0.54, 0.74, 0.83, 0.94
	10%-H_2SO_4 Reagent	0.11, 0.34, 0.49, 0.57, 0.63, 0.74, 0.80, 0.86, 0.94

Discussion

This study was carried out on the barks of *Dillenia ovata* Wall. ex Hook.f. & Thomson which revealed that the phytochemical constituents including alkaloids, phenolic compounds, tannins, flavonoids, coumarins, cardiac glycosides, terpenoids, resins, saponins, and polypeptides, except for essential oils, are present in the plant and the results were summarized in Table 1. The phytochemicals, either alone and/or in combination, have tremendous therapeutic potential in curing various ailments caused by inflammation, allergy, reactive oxygen species, fungi, bacteria, viruses and other pathogens (Prakash et al., 2012).

Barcelo (2015) reported the presence of terpenoids, flavonoids, cardiac glycosides, tannins, and polyphenol in the *Dillenia* spp. This is in accordance with our study showing the positive test of

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phenolic compounds, tannins, flavonoids, and terpenoids. These phytochemicals function as dietary fiber, antioxidants, anticancer, detoxifying agents, immunity-potentiating agents and neuropharmacological agents (Saxena et al., 2013). Moreover, the cardiac glycosides and the saponins were positively tested in the barks of *Dillenia ovata* Wall. ex Hook.f. & Thomson. This finding is comparable with the report of Bose et al. (2010) demonstrating the presence of glycosides and saponins in *Dillenia indica* Linn. Cardiac glycosides are well known to exert their therapeutic effects on various diseases such as heart failure, atrial arrhythmia, and cancers (Prassas & Diamandis, 2008). The plant-derived saponins have been proved the efficacy in the treatment of human diseases as anti-inflammatory, hypocholesterolemic and immune-stimulating agents (Tamura et al., 2012).

Several reports indicated the presence of alkaloids in the *Dillenia* spp. (Amritveer et al., 2016), which is in agreement with our study. Alkaloids in plants are one of the largest groups of plant secondary metabolites encompassing neuroactive molecules, such as caffeine and nicotine, as well as life-saving medicines including emetine used to fight oral intoxication and the antitumoral vincristine and vinblastine (Matsuura & Fett-Neto, 2015). Besides, phytochemical evaluation showed that the barks of *Dillenia ovata* Wall. ex Hook.f. & Thomson possess coumarins, resins and polypeptides which is in accordance with Lima et al. (2014), indicating that these phytocomponents of Dilleniaceae plants express anti-ulcer, antiinflamatory, immunological and antibacterial activities.

The TLC profiling of the ethanolic extract of *Dillenia ovata* Wall. ex Hook.f. & Thomson barks gave an impressive separation of the phytochemicals with different R_f values reflecting an idea about their polarity. Biswas & Pandita (2015) revealed a similar finding of TLC fingerprints expressing different R_f values of the alcoholic extract of the *Dillenia* spp., under the UV detection of 254 and 366 nm.

Conclusion

This study reports the first discovery of phytoconstituents including alkaloids, phenolic compounds, tannins, flavonoids, coumarins, cardiac glycosides, terpenoids, resins, saponins and polypeptides in the ethanolic extract of *Dillenia ovata* Wall. ex Hook.f. & Thomson barks. This plant's TLC layouts of the spots detected under 254-366 nm UV light and 10%-H_2SO_4 reagent give good separation with the mobile phase system of Chloroform:Ethanol (15:1). These reveal the authentication and the standardization for the quality control of *Dillenia ovata* Wall. ex Hook.f. & Thomson and exhibit significant benefit for the future research in term of natural drug isolation.

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Declaration of Conflicts of Interest

No conflict of interest associated with this work.

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