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Research Article

### **Phytochemical and thin layer chromatography analyses of *Morinda citrifolia* L. of Tboung Khmum Province, Cambodia**

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#### **Abstract**

*Morinda citrifolia* L. has been used for the treatment of such diseases as gastritis, dermatitis, respiratory infections, menstrual and urinary disorders, fever and diabetes. This study aims to identify the phytochemical constituents of the ethanolic extracts of *Morinda citrifolia* L. and to record its profile based on Thin Layer Chromatography (TLC). The phytochemical analysis of the ethanolic extracts of *Morinda citrifolia* L. indicated that the roots contained phenols, coumarins, cardiac glycosides and steroids. The fruits possessed alkaloids, phenols, tannins, coumarins, cardiac glycosides and steroids. The leaves consisted of alkaloids, saponins, phenols, tannins and coumarins. Their layouts of TLC analysis investigated under 254-366 nm UV light gave good separation with the mobile phase system of chloroform:methanol (7:3). These reveal authentication for the quality control of *Morinda citrifolia* L. and exhibit significant role in the fields of drug isolation and medicinal chemistry.

**Keywords:** medicinal plants, Tboung Khmum, Cambodia, Rubiaceae, *Morinda citrifolia* L.

#### **Introduction**

Cambodia is located in Southeast-Asia where the natural resources are well known. Owing to its diversification, numerous types of medicinal plants have been traditionally used for centuries ago, and it remains practiced until today (Chassagne et al., 2016). *Morinda citrifolia* L. is one of the most common medicinal in Cambodia. Its therapeutic activities are generally embedded in different parts of roots, stems, leaves and fruits (Krishnakumar et al., 2015). *Morinda citrifolia* L., commonly called 'noni' in family Rubiaceae, has been applied to prevention and treatment of various diseases relating to skin, brain, gastrointestinal tract, heart, liver and cancer (Ullah et al., 2016). The phytochemicals of *Morinda citrifolia* L. leaves, fruits and roots have been reported to have such biological activities as



larvicidal, antioxidant, antimicrobial, anticarcinogenic, antifungal, antibacterial, anti-inflammatory and antiviral effects (Assi et al., 2015). Several studies reported its phytochemicals including alkaloids, phenols, flavonoids, glycosides, tannins, saponins and phytosterols (Nagalingam et al., 2012; Valli & Murugalakshmi, 2014; Kochuthressia & Jaseentha, 2015). Thin layer chromatography (TLC) is the most widely used method for the characterization of medicinal plants, and it is used as a “fingerprint” method for the description of the chemical components in plant extracts (Gocan & Cimpan, 2004). TLC fingerprinting of *Morinda citrifolia* L. has been reported to give a good separation under the solvent systems of chloroform, ethyl acetate and methanol (Satwadhhar et al., 2011). However, there has not been any scientific evidence on the chemical constituents of this plant yet in Cambodia although it has been widely used by the Cambodian people for years. Thus, this study was conducted to identify the phytochemicals of the ethanolic extract of *Morinda citrifolia* L. roots, fruits and leaves, and to record its TLC fingerprint.

## Materials and Methods

*Preparation of plant materials:* The whole *Morinda citrifolia* L. plant, authenticated by University of Puthisastra (UP)-Herbarium (UPFPT-250001), was collected from Sone Prammuy Village, Chup Commune, Tboung Khmum District, Tboung Khmum Province, Cambodia (11°55'37.8"N 105°36'20.7"E) in December of 2016 (Figure 1). Its roots, leaves and fruits were separated and cut into small pieces, and they were subjected to dryness by exposing to air in room temperature. Through maceration method, the dried plants were extracted with ethanol for 48 h in the room temperature in Pharmacognosy Laboratory, Department of Pharmacy, Faculty of Pharmacy, UP.

*Phytochemical analysis:* The ethanolic extract of roots, fruits and leaves of *Morinda citrifolia* L. underwent phytochemical screening in order to detect the presence (or absence) of alkaloids, saponins, flavonoids, tannins, coumarins, cardiac glycosides and steroids (Nagalingam et al., 2012; Roopalatha & Nair, 2013; Djaafar & Ridha, 2014; Kochuthressia & Jaseentha, 2015).

*Test for alkaloids:* 2 ml of ethanolic extracts were stirred with 2 ml of 1%-HCl in the steam bath and filtered. The filtrate was transferred and divided into four test tubes with 1 ml each. Few drops of Meyer's, Wagner's and Dragendorff's reagents were added into each tube. One control tube was left without reagents. The result is positive when precipitate is present.

*Test for saponins:* 2 ml of ethanolic extracts were added with 5ml of distilled water in the test tube, and shaken strongly until there is the formation of froth. The result is positive when froth persisted for 10 min.



Figure 1: *Morinda citrifolia* L.



*Test for phenols:* 2 ml of ethanolic extracts were added with few drops of Ferric chloride 10%. Blue or green color precipitate indicates the presence of phenols.

*Test for tannins:* 2 ml of ethanolic extracts were stirred with 1 ml of distilled water and filtered, and a few drop of ferric chloric were added to the filtrate. Formation of blue color indicates the presence of tannins.

*Test for coumarins:* 2 ml of ethanolic extracts were added to the test tube. It was added with 1N NaOH. Filter paper was used to cover over the mouth of the tube; it was boiled in the water bath. The filter paper was removed and examined under UV light. The yellow florescence indicates the presence of coumarins.

*Test for cardiac glycosides:* 5 ml of ethanolic extracts were mixed with 2 ml of glacial acetic acid and solution  $\text{FeCl}_3$ . The mixture was added with 1ml of concentrated  $\text{H}_2\text{SO}_4$ . The formation of blue color in acetic acid layer confirms the test.

*Test for steroids:* 2 ml of ethanolic extracts was mixed with 2ml of chloroform and 2ml of  $\text{H}_2\text{SO}_4$  consequently and slowly. The positive result is the development of a red and green layer.

*Thin layer chromatography:* 1 g of dried plants was added with 100 ml ethanol and macerated for 48 h before filtration. 20 $\mu\text{l}$  of the filtrate was used to spot on TLC plate. TLC Silica gel 60  $\text{F}_{254}$  of Merck was subjected to the TLC analysis. Mobile phase of ratio: Chloroform:Methanol (7:3) was formulated. CAMAG UV Lamp with 254-366 nm wavelengths was used for the detection of component spots onto the TLC plate.

## Results

The ethanolic extracts of *Morinda citrifolia* L. roots revealed the presence of alkaloids, phenols, coumarins, cardiac glycosides and steroids; fruits showed the positive tests of alkaloids, phenols, tannins, coumarins, cardiac glycosides and steroids; and leaves indicated the existences of alkaloids, saponins, phenols, tannins and steroids (Table 1).

Table 1: Phytochemical analysis of ethanolic extracts of roots, fruits and leaves of *Morinda citrifolia* L.

Phytochemical Constituents	Plant Parts of <i>Morinda citrifolia</i> L.		
	Roots	Fruits	Leaves
Alkaloids	-ve	+ve	+ve
Saponins	-ve	-ve	+ve
Phenols	+ve	+ve	+ve
Tannins	-ve	+ve	+ve
Coumarins	+ve	+ve	+ve
Cardiac glycosides	+ve	+ve	-ve
Steroids	+ve	+ve	-ve

Note: (+ve) represents the positive result; (-ve) represents the negative result.

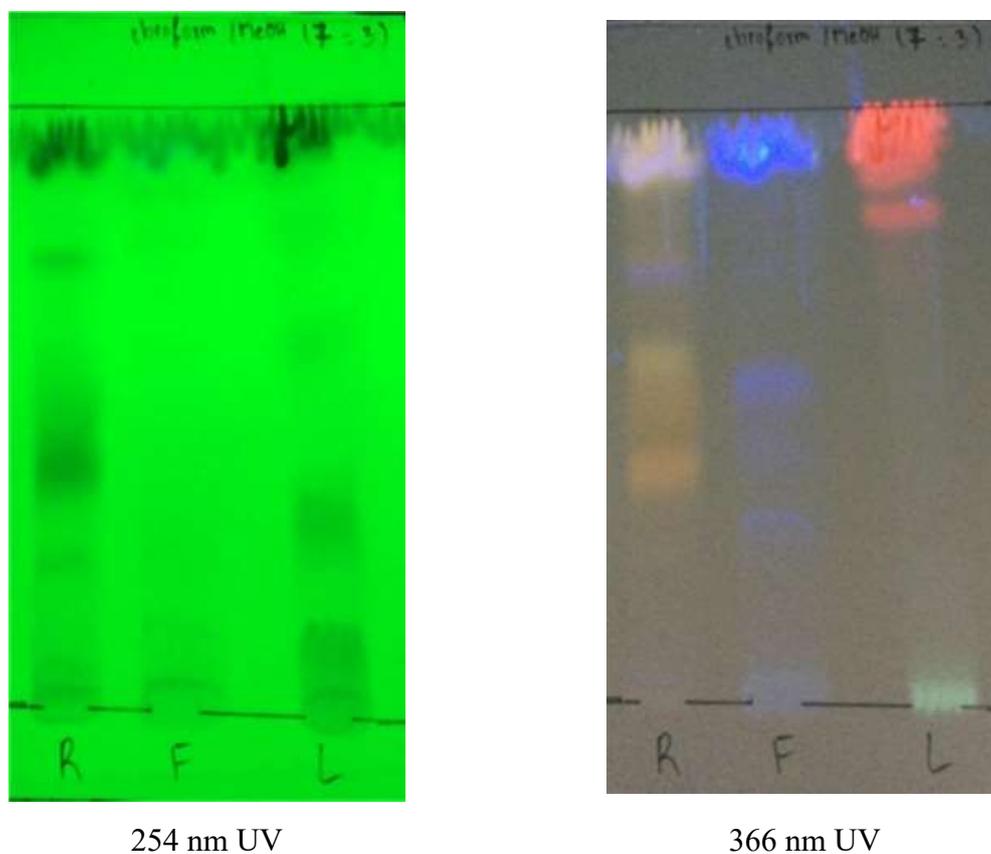


Figure 2: TLC analysis of ethanolic extracts of roots, fruits and leaves of *Morinda citrifolia* L. under the mobile phase of Chloroform:Methanol (7:3). Note: R: roots, F: fruits, L: leaves.

Table 2:  $R_f$  values of ethanolic extracts of roots, fruits and leaves of *Morinda citrifolia* L. detected by 254-366 nm UV

Plant Parts of <i>Morinda citrifolia</i> L.	Mobile Phase: Chloroform:Methanol (7:3)	
	$R_f$ values under 254 nm UV	$R_f$ values under 366 nm UV
Roots	0.07, 0.25, 0.38, 0.47, 0.56, 0.74, 0.83	0.25, 0.38, 0.49, 0.58, 0.74
Fruits	0.14, 0.76, 0.85	0.29, 0.42, 0.54
Leaves	0.13, 0.33, 0.63, 0.82	0.71, 0.83

## Discussion

Dandge et al. (2016) reported positive tests of alkaloids, tannins and coumarins in the ethanolic extract of leaves of *Morinda citrifolia* L. The phenols and saponins in ethanolic extract of *Morinda citrifolia* L. leaves have also been recorded by Kochuthressia & Jaseentha (2015). These are in agreement with our results displaying the availability of alkaloids, tannins, coumarins, phenols and saponins in leaves



of *Morinda citrifolia* L. extracted with ethanol. The fruits of *Morinda citrifolia* L., extracted with ethanol, have been found to contain alkaloids, phenols, tannins, cardiac glycosides and steroids (Nagalingam et al., 2012), which are consistent with our observation. Moreover, this study showed the presence of coumarins in the ethanolic extract of fruits of *Morinda citrifolia* L. This is similar to the report of Valli & Murugalakshmi (2014) revealing the positive analysis of coumarin glycosides in the ethanolic extract of *Morinda citrifolia* L. fruits. This investigation showed the positive analysis of phenols, cardiac glycosides and steroids in the ethanolic extract of roots of *Morinda citrifolia* L. which is in accordance with Sibi et al. (2012). Besides, coumarin tested positive in the ethanolic extract of *Morinda citrifolia* L. roots, which is similar to the records of Nerurkar et al. (2015). TLC analyses of roots, fruits and leaves of *Morinda citrifolia* L. , under the mobile phase condition of Chloroform:Methanol (7:3) showed good separation of spots onto the TLC layout detected by 254-366 nm UV light, which is validated by several reports (Potterat et al., 2007; Satwadhar et al., 2011; Alwala et al., 2014).

## Conclusion

Collectively, this study concludes the phytochemicals of the ethanolic extracts of root, fruit and leaf parts of *Morinda citrifolia* L. Phenols and coumarins are main components in these three parts of *Morinda citrifolia* L. The TLC layouts of the spots detected under 254-366 nm UV light give good separation with the mobile phase system of Chloroform:Methanol (7:3). These reveal authentication for the quality control of *Morinda citrifolia* L. and exhibit significant role in the fields of drug isolation and medicinal chemistry.

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## Declaration of conflict of interest

No conflict of interest associated with this work.

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Asian Journal of Pharmacognosy (2017) 1(3):5-11

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