HPTLC fingerprint profile of *Ardisia elliptica* Thunb leave

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ABSTRACT

To develop the finger print of medicinally and economically important leaves of *Ardisia elliptica* Thunb. Ethanol extract of the leaves were developed in the mobile phase of Ethyl acetate: Formic acid: Acetic acid : Water (100 : 11 : 11 : 27) using standard procedures and scanned under UV at 254 nm, 366nm and under visible light. Results: The HPTLC fingerprinting of the ethanol extract has shown several peaks with different Rf values. 2 µL and 4 µL of ethanol extract showed 10 spots while 6 µL and 8 µL has shown 11 spots in the above said solvent system. This finger print would be helpful in the identification and authentication of this species.

Keyword: HPTLC, Thin-layer chromatography, *Ardisia elliptica* Thunb, medicinal plants, fingerprinting

INTRODUCTION

The marketing of plants are very important items of trade in many parts of the world. The plants use these for a variety of purposes such as food, cosmetics, flavors, spices, and medicines. It seems that plants, medicinal purposes, are the most common category. While medicinal plants are found to be safe and effective but synthetic drugs causes side effects. Hence people are more favors to use natural products obtained from medicinal plants. Even today products and compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries. Phytochemical analysis of plants, used in folklore medicine, has yielded a number of active ingredients with various biological activities. Plants are rich in a wide variety of secondary metabolites such as phenolic acids, terpenoids, alkaloids, flavonoids etc. The plants are distinct not only in their pharmacological properties but also in a variety of morphological characters, including those of root, stems, leaves, flower, *etc*. (Uncle, 2007, Alvin *et al*., 2014, Arya *et al*., 2014, De Boer *et al*., 2014, Lahlou, 2013, Duffy *et al*., 2012, Mishra. and V.K., 2011, Mukhtar *et al*., 2008, Street *et al*., 2008, Lam, 2007, Gurib-Fakim, 2006 and Balunas *et al*., 2005). The main limitation in the use of traditional remedies is the lack of standardization of plant material, plants products, manufacturing process and the final product. Thus quality control for plant materials preparations or plants products is the need of the day.

However, it is much more difficult than synthetic drugs because of the chemical constituents of the ingredients any loss in a particular chemical may result in loss of pharmacological activities of that medicinal plants. If identification of an active principle is not possible, a characteristic of chemical constituent e.g. chromatographic fingerprint should be identified to confirm consistent quality. Analytical separation techniques, like High performance liquid Chromatography (HPLC), Gas Chromatography (GC) and Mass Spectrometry (MS) were among the most popular methods of choice used for quality control of plant materials and finished plants products, nevertheless High performance thin layer chromatography (HPTLC) is a priceless tool for decisive standardization. It can provide chromatographic fingerprints that can be visualized and stored as electronic images where they may be used as reference guides for future standardization (Hosu *et al*., 2015, Guzelmeric *et al*., 2015, Mohammed *et al*., 2014, I. *et al*., 2014, Gopal *et al*., 2014, Gallo *et al*., 2014, Patil *et al*., 2013, Alajmi and Alam. 2013, Ojha and Kumar. 2012, Michel*et et al*., 2012, Hussain *et al*., 2012, Gunalan *et al*., 2012, Gomathi *et al*., 2012, Mariswamy *et al*., 2011, Vermaak *et al*., 2010, Rasheed *et al*., 2010, Lobo *et al*., 2010 and Suryavanshi *et al*., 2007). Currently HPTLC has become as an alternative to HPLC standardization of plant materials and finished plants products by reason of its simplicity, accuracy, rapidity, cost-effectiveness, high sample throughput and need for only minimum sample clean up. The main benefit of HPTLC is that many samples can be run simultaneously using a little volume of mobile phase, thus reducing time and cost per analysis (Guzelmeric *et al*., 2015, Loescher *et al*., 2014, Tian *et al*., 2009, Akowuah *et al*., 2006 and Kaul, 2005).

Ardisia elliptica Thunb. (family Myrsinaceae), also known in Thailand as Ram Yai or Pilangkasa, is a native medicinal plant commonly found in the Southeast Asia including Thailand. It is glabrous shrub or small tree to 5 m tall with smooth stems and new foliage often reddish and Fruit a rounded drupe, 6 mm wide, red turning to black when ripe, with white juicy flesh (Center for invasive species and ecosystem health, 2007, United States Departmant of Agriculture, 2004). In traditional medicines, the Malaysians used the decoction of the leaves to treat chest pain, herpes and measles (Ahmad *et al*., 2002, Burkill, 1966). The crude extract and some phytochemical constituents from *A. elliptica* were proven to possess various biological activities such as antiplatelet activity, antibacterial activity, antioxidant activity, anti-proliferative activity, antiviral activity (Ching *et al*., 2010, Phadungkit *et al*.,2006, Kobayashi *et al*., 2005, Yen, 2005, Moongkarndi *et al*., 2004, Jalil *et al*., 2004 and Chiang *et al*., 2003). Previously reported phytochemical constituents from the leaves of *A. elliptica* are α-amyrin, β-amyrin, bauerenol, bergenin, rapanone,5-(Z-heptadec-4-enyl) resorcinol, 5-pentadecylresorcinol (Jalil *et al*., 2004, Liu *et al*.,1993, Chow *et al*., 1991 and Ahmad *et al*., 1977) .While, more work needs to be undertaken to fully characterize these compounds, identify the molecules with bioactive roles. Therefore, the current study was aimed to determine the chromatographic finger print profile of *A. elliptica* leaves. The data obtained in the present study could be useful in proper identification and authentication of this plant which is a primary pre-requisite for standardization and recognition of herbal medicines.

MATERIAL AND METHODS

A Camag HPTLC system(Muttenz, Switzerland) equipped with an automatic TLC sampler 4 (ATS 4, connected to a nitrogen tank), TLC scanner 3 (WINCATS version 1.3.4), and UV cabinet (Reprostar 3) was used for the analysis

All the solvents were of analytical grade from Merck (Darmstadt, Germany). Silica gel 60 F_{254} HPTLC precoated plates were procured from Merck (Darmastadt, Germany)

The samples of *A. elliptica* was collected from its natural habitat, Thailand in January, 2013. The samples were identified by comparison with the plant specimens at the Herbarium Section, Forestry Department, Bangkok, Thailand

The leaves of *A. elliptica* was separated from the stems, washed thoroughly and dried in an oven at 50 ºC. The dried sample was ground to powder. The powder was extracted with ethanol, filtered and concentration under vacuum to obtain the crude extract. Two hundred milligram of the accurately weighed extract was transferred to a 10 mL volumetric flask. Methanol was added to volume (final concentration of 20,000 µg mL^{-1}).

The Ethyl acetate: Formic acid: Acetic acid: Water in the proportion 100: 11:11: 27 (v/v)

Chromatography was performed on HPTLC plates (20 cm × 10 cm) pre-coated with silica gel 60 F_{254}. 0.2 mm. layer thickness (Merck, Darmstadt, Germany). Samples were spotted as 8 mm bands, started 25 mm from the left edge, 10 mm from the bottom edge and 16.6 mm of track distance, using a Camag TLC sampler 4 sample applicator under a flow of N_2 gas. The plate was developed to a distance of 8 cm in a Camag twin through chamber previously equilibrated with the mobile phase (45 min). The developed HPTLC plate was dried using a dryer for 2 min. The developed HPTLC plate was captured the images under UV light at 254 and 365 nm and the plate which was scanned between 200 and 400 nm, with a TLC scanner 3 having a deuterium lamp in conjunction with Cats 1.3.4 version software. The wavelength of 350 nm (λ_{max}) was used for quantitation. The chromatograms were recorded.

Results

The Chromatograms shown in Fig. 1 indicate that all sample constituents were clearly separated without any tailing and diffuseness. It is evident from Table 1 that in 2 µL of ethanol extract of *A. elliptica* leave, there are 10 spots at the following Rf 0.02, 0.13, 0.19, 0.26, 0.40, 0.44, 0.54, 0.63, 0.68, 0.88, as shown in Fig. 2, indicating the occurrence of at least 11 different components in 2 µL of ethanol extract. Out of 10 components, the component with Rf values 0.02, 0.44, 0.54, 0.63 and 0.88 were found to be more predominant as the percentage area was more with 10.10 %, 17.32 %, 10.10 %, 22.61 % and 12.24 % respectively. The remaining components were found to be very less in quantity as the percent area for all the spots were less than 8.0%.

It is revealed from Table 1 that in 4 µL of ethanol extract of *A. elliptica* leaves there are 10 spots as shown in Fig. 2 indicating the occurrence of at least 10

different components in ethanol extract. Out of 10 components, the component with Rf values 0.02, 0.12, 0.40, 0.44, 0.54, 0.62 and 0.88 were found to be more predominant as the percentage area was more with 9.07%, 9.33%, 8.47%, 16.99%, 10.43, 20.59 and 9.12% respectively. The remaining components were found to be very less in quantity as the percent area for all the spots were less than 8%.

Table 1 shows that in 6µL of ethanol extract of *A. elliptica* leaves there are 11 spots as shown in Fig. 2 indicating the occurrence of at least 11 different components in ethanol extract. Out of 11 components, the component with Rf values 0.01, 0.12, 0.40, 0.44, 0.54, 0.62 and 0.88 were found to be more predominant as the percentage area was more with 8.05%, 9.70%, 8.73%, 16.71%, 10.87% , 19.46% and 9.87% respectively. The remaining components were found to be very less in quantity as the percent area for all the spots were less than 8 %.

Table 1 indicate that in 8µL of ethanol extract, there are 11 spots at the following Rf 0.01, 0.12, 0.20, 0.25, 0.39, 0.43, 0.54, 0.62, 0.68, 0.78, 0.88 as shown in Fig.2 indicating the occurrence of at least 11 different components in ethanol extract. Out of 11 components, the component with Rf values 0.12, 0.39, 0.43, 0.54, 0.62, 0.68 and 0.88 were found to be more predominant as the percentage area was more with 8.06%, 8.84%, 14.70%, 10.76%, 19.09 %, 9.03 and 12.25% respectively. The remaining components were found to be very less in quantity as the percent area for all the spots were less than 6.1%.

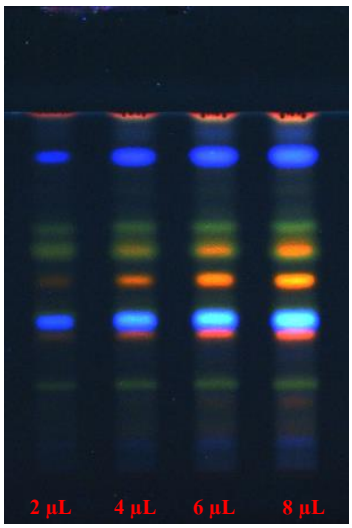

Figure 1 HPTLC chromatogram of ethanol extract of *A. elliptica* leaves.

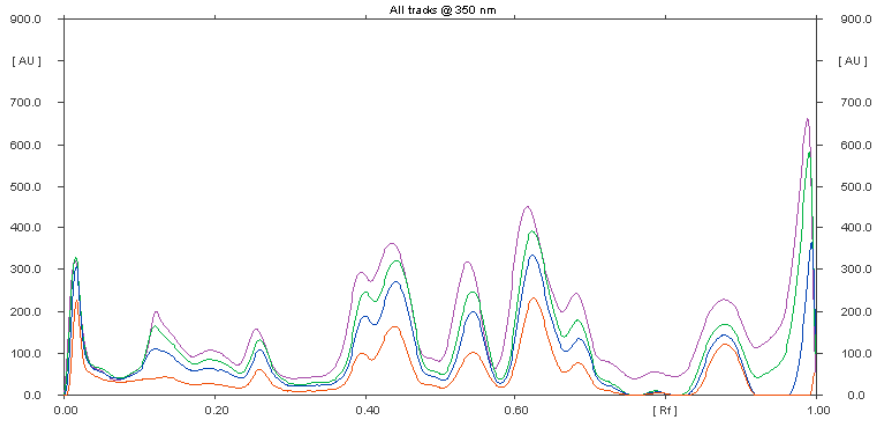

Figure 2 Fingerprint of ethanol extract of *A. elliptica* leaves.

Spot volume	Peak	Max Rf	Max Height (AU)	Area (%)
2 µL	1	0.02	227.3	10.10
	2	0.13	44.0	7.34
	3	0.19	29.0	2.78
	4	0.26	62.9	4.53
	5	0.40	100.5	7.24
	6	0.44	164.5	17.32
	7	0.54	102.5	10.01
	8	0.63	231.3	22.61
	9	0.68	77.4	5.84
	10	0.88	121.9	12.24
4 µL	1	0.02	306.6	9.07
	2	0.12	111.2	9.33
	3	0.19	64.9	4.36
	4	0.26	109.7	5.13
	5	0.40	189.4	8.47
	6	0.44	270.9	16.99
	7	0.54	199.7	10.43
	8	0.62	333.3	20.59
	9	0.69	135.1	6.51
	10	0.88	144.4	9.12

Table 1 Peak list and Rf values of the chromatogram of ethanol extract of *A. elliptica* leaves.

Spot volume	Peak	Max Rf	Max Height (AU)	Area (%)
6 µL	1	0.01	328.6	8.05
	2	0.12	164.9	9.70
	3	0.19	86.6	4.49
	4	0.26	131.9	5.02
	5	0.40	246.2	8.73
	6	0.44	320.8	16.71
	7	0.54	247.3	10.87
	8	0.62	391.1	19.46
	9	0.68	180.2	6.84
	10	0.79	12.2	0.25
	11	0.88	170.2	9.87
8 µL	1	0.01	321.4	6.09
	2	0.12	200.0	8.06
	3	0.20	107.4	4.26
	4	0.25	158.7	5.03
	5	0.39	293.3	8.84
	6	0.43	362.1	14.70
	7	0.54	318.8	10.76
	8	0.62	449.9	19.09
	9	0.68	244.7	9.03
	10	0.78	56.7	1.89
	11	0.88	228.7	12.25

Table 1 (cont) Peak list and Rf values of the chromatogram of ethanol extract of *A. elliptica* Thunb leaves.

DISCUSSION

Thus the developed chromatogram will be specific with selected solvent system of Ethyl acetate: Formic acid: Acetic acid: Water (100 : 11 : 11 : 27), Rf value and fingerprint profile of *A. elliptica* leave serve the better tool for standardization of the raw material of *A. elliptica*. Characteristic HPTLC fingerprinting of particular plant species will not only help in the identification and quality control of a particular species but also provide basic information useful for the isolation, purification, characterization and identification of marker chemical compounds of the species. Thus the present study will provide sufficient information about identification, standardization and quality control of medicinal plant. The quality of herbal medicines is defined in terms of the content of its bioactive compounds. Hence, HPTLC fingerprint profile of herbal products is such an important and powerful procedure which has often been employed for the determination of bioactive components of the

herbal medicine. Such finger printing is useful in quality control of herbal products and checking for the adulterant. Therefore, it can be useful for the evaluation of different marketed pharmaceutical preparations and plant systematic studies

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CONCLUSION

In conclusion, the results obtained from qualitative evaluation of HPTLC fingerprint images will be helpful in the identification and quality control of the plant materials preparations or plants products and ensure therapeutic efficacy.

The HPTLC fingerprints will help the manufacturer for quality control and standardization of plant materials and products. Such finger printing is useful in differentiating the species from the adulterant and act as biochemical markers for this medicinally important plant in the pharmaceutical companies and plant systematic studies.

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