Comparative Phytochemistry of *Physalis angulata* L. (Family Solanaceae)

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ABSTRACT

The morphology of *Physalis angulata* L. from Kasetsart University and Ao Khoei, Phangnga Province is different. A comparative phytochemical study of lipophilic crude extracts from the aerial part (leaves and stem) and underground part (root) has been conducted on plants from two different habitats. TLC screening with spraying colour detection reagent including Dragendorff's reagent, anisaldehyde - sulfuric acid reagent, 10% NaOH and 20% H_2SO_4 in order to detect alkaloids, terpenoids and steroids, coumarins and general organic compounds respectively. The results showed that the extracts from all different plant parts of *P. angulata* collected from two different habitats contained alkaloids, terpenoids, steroids, coumarins. The chemical profiles by using HPLC analysis showed the different between the same plant parts (aerial part and underground part) collected from different habitats.

Key words: *Physalis*, Phytochemistry, TLC, HPLC

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INTRODUCTION

Throughout the world, a wide variety of natural products have been used for their high efficiency, safety and minimal side effects on human and the environment. Thailand has a rich biological diversity. However, the loss of some natural resources has occurred. *Physalis angulata* L. is an annual herb, native to tropical America but now distributed as a weed to many parts of the tropical areas of Africa, Asia and America (Bastos *et al*., 2008), including Thailand. Many parts of this plant have been used in various countries as popular medicine for treatment of various illnesses (Lin *et al*., 1992). This species is common plant in the country, and even weeds that seem to be useless may play an important role to reduce other disadvantage weeds in the future.

P. angulata belongs to the family Solanaceae. It is known by different Thai names, thong theng (Central), tom tok (Chiang Mai) or pung ping (Peninsular) (Smitinand, 2001). It is an annual herb, branched stems, pale yellow or white corolla and spotted in throat, fruiting calyx ovoid (Zhi-yun *et al*., 1994). Extracts or infusions from this plant have been used in various countries in popular medicine as a treatment for a variety of illness, such as malaria, asthma, hepatitis, dermatitis, liver problems and rheumatism, as well as having anticancer, antimycobacterial, antileukemia, antipyretic properties and as an immunomodulatory agent and diuretic (Lin *et al*., 1992; Chiang *et al*., 1992)

Phytochemistry

Phytochemically, *P. angulata* is distinguished by the presence of several steroid lactones, in general belonging to the physaline and withanolide type. The aerial parts of *P. angulata* yielded physalin B and closely related compounds (Subramanian and Sethi, 1970). In a comparative study of two varieties, the stem of *P. angulata* raised from seeds procured from Lucknow yielded, on ethanolic extraction, four physalins; physalin E, F, G and J. The stem of the plant, raised from seeds obtained from Copenhagen gave physalins B, E, F, H, I and J. The leaves of the plant from Copenhagen seeds yielded physalins B, E and F. (Row *et al*., 1978a, 1978b). Investigation of the cytotoxic flavonol glycoside constituents in methanol extract of the leaves of *P. angulata* from Texas, USA led to the isolation of myricetin 3-O-neohesperidoside (Ismail and Alam, 2001). The investigation of the cytotoxic withanolindes, isolated from the MeOH extract of the aerial parts of this plant, led to isolation of four new withanolindes, physagulins L-O. Their structures were determined by spectroscopic techniques (He *et al*., 2007). Phytochemical investigation of *P. angulata* was initiated following primary biological screening. Fractionation of $CHCl_3$ and n-BuOH soluble of the MeOH extract from the whole plant was guided by in vitro cytotoxic activity and led to the isolation of seven new withanolides, withangulatins B-H, and a new minor physalin, physalin W (Damu *et al*., 2007). Withangulatin A, withanolide from *P. angulata* shows immunosuppression effect via heme oxygenase 1-dependent pathways (Sun *et al*.,

2011). A recently study reported the discovery of three antiproliferative withanolides, namely physangulidines A, B and C, in *P. angulata.* Their structures were determined by a combination of HRMS, NMR spectroscopic and X-ray crystallographic methods (Jin *et al*., 2011). Phygrine, the pyrrolidine alkaloid, was isolated from the roots and aerial parts of this plant (Basey *et al*., 1992). In the present study we are comparing the chemical of *P. angulata* from two different habitats by HPLC analysis and TLC screening.

MATERIALS AND METHODS

1. Plant Identification

Three parts of *P. angulata* (leaves, stem and root part) utilized in this study were collected from Kasetsart University and two parts (aerial part and root part) utilized in this study were collected from Ao Khoei, Phangnga Province. Botanical identification was achieved through comparison with specimens deposited in The Forest Herbarium (BKF).

2. Plant Extraction

Air-dried samples were powdered and extracted in methanol for 7 days in the dark at room temperature, then the extracts were filtered through Whatman no. 1 filter paper and subsequently concentrated by using a rotary evaporator at 37° C afforded semi-solid crude extract. The concentrated crude extract was successively partitioned into two parts: hydrophilic extract and lipophilic crude extract with distilled water and chloroform respectively. The lipophilic crude extract was then evaporated into dryness for further experiments.

Phytochemical Screening (TLC: Thin Layer Chromatography)

Phytochemical screening of secondary metabolites i.e. alkaloids, terpenoids, steroids, coumarins and organic compound was done by thin layer chromatography (TLC). Chromatography was performed on 10 x 20 cm silica gel TLC plates (0.2 mm thickness, 60 F_{254}, Merck) plates. The lipophilic extracts of samples at concentration 10 mg/ml were spotted 20 drops/spot on TLC plates. The TLC plates were developed on solvent system n-hexane-ethy acetate-methanol 3:1.5:0.5 (v/v). For complete development TLC plates when solvent move distance 15 cm from start of extract spots. The developed TLC plates were dried and sprayed by colour detection reagents. Dragendroff's reagent was used for screen alkaloids. Orange colour spot indicates the presence of alkaloids (Merck, 1980). Anisaldehyde sulfuric acid reagent detected terpenoids and steroids. Violet, blue, red, grey or green spot indicates the presence of terpenoids and steroids (Merck, 1980). 10% NaOH examined for coumarins, blue or yellow-green fluorescence of spot under UV 365 nm indicate the presence of coumarins (Farnsworth, 1966). The general organic substances were detected by spraying 20% H_2SO_4 in ethanol. Yellow, grey or black colour of spot indicates the presence of the general organic

substances (Merck, 1980). The relative between distance compounds move in chromatography to solvent was calculated to give the relative front (R_f) value.

$$R_f = \frac{\text{Distance moved by the solute (cm)}}{\text{Distance moved by the solvent (cm)}}$$

HPLC (High Performance Liquid Chromatography)

10 mg samples in methanol (HPLC grade) 1 ml were prepared. The HPLC analysis was undertaken on Agilent 1100 series, UV diode arry detection at 230 nm by Reisch's Method.

RESULTS AND DISCUSSION

Morphological Study

The morphology of *P. angulata* collected from two different habitats, Kasetsart University (KU) and Ao Khoei, Phangnga Province (PN) are similar, however there are some different. The leaves at KU are ovate shape and there have margins irregular teeth but at PN the leaves are lanceolate with entire margins. The *P. angulatata* from KU has many more branches and there are longer than those from PN. (Table 1)

Table 1 Morphology of *P. angulata* from KU and PN

Localities	Habit		Leaves		Flower		Fruiting-calyx	Seed
	Tall	Branches	Shape	Margin	Corolla	Anther		
KU	>50 cm	many	ovate	irregular	pale- yellow	Pale blue	green with-purple ribs	lens-shape
PN	<50 cm	less	lanceo-late	entire	yellow	Pale blue	green with-purple ribs	lens-shape

TLC screening

Preliminary phytochemical investigation of lipophilic extracts of *P. angulata* from Kasetsart University and Ao Khoei revealed the presence of alkaloids, terpenoids, steroids, coumarins and the general organic substances. Alkaloids detected by Dragendroff's reagent had R_f values 0.53. There were 9 different R_f values of terpenoids and steroids observed in five lipopophilic extracts in the range of 0.06 to 0.96. Anisaldehyde sulphuric acid reagent was used to detect them. Eight different R_f values of coumarins in the range of 0.13 to 0.89 were observed in the lipophilic extracts. They were detected by 10% NaOH and exposed fluorescence under UV light. For the general organic

substances, the TLC plate was sprayed with 20% H_2SO_4 in ethanol. The result showed that leaves, stem from Kasetsart University and aerial part from Ao Khoei detected the general organic substances in the range of R_f values between 0.64 to 0.86. The phytochemical profiles of lipophilic extracts in this study are presented in Table 2.

Table 2 Phytochemical profiles of lipophilic extracts of *P. angulata* from KU and PN

R_f values	Leaves KU	Stem KU	Root KU	Aerial part PN	Root part PN
0.03	-	+3	+3	-	+3
0.06	+2	+2	+2	+2	+2
0.10	+2	+2,+3	+2,+3	+2,+3	+2,+3
0.24	+2	+2	+2	+2	+2
0.53	+1	+1	-	+1	-
0.56	-	-	-	-	+3
0.62	-	-	-	-	-
0.63	+3	-	-	-	-
0.64	+4	-	-	-	-
0.70	-	-	-	+4	-
0.73	+2	+2	+2	+2	+2
0.74	-	-	-	-	+3
0.80	+4	-	+3	-	+3
0.83	+2	+2	+2	+2	+2
0.84	-	-	+3	-	-
0.86	+2,+4	+4	+3	+2,+4	+2,+3
0.89	+3	+3	+3	-	+3
0.90	-	-	-	+2	+2
0.93	+2	+2	+2	+2	+2
0.96	+2	+2	+2	+2	+2

+ = present; 1= alkaloids; 2= terpenoids and steroids; 3= coumarins; 4= the general organic substances

HPLC Analysis

Result of HPLC analysis at 230 nm of *P. angulata* extracts from Kasetsart Universtiy (KU) and Ao Khoei, Phangnga Province (PN), shows presence of various constituents as evidenced by the chemical profiles obtained at various retention times.

Comparison of chemical profiles of the different plant parts collected from the same habitat

For this result, we have compared the parts of the same plant from Kasetsart University (KU). Leaves (KU) extract had fewer peaks than root (KU) extract, leaves (KU) extract had three dominant

peaks and there were five dominant peaks for root (KU) extract. Stem (KU) extract and root (KU) extract both had six dominant peaks (Figure 1-2). Comparison of different plant parts of the same plant collected from Ao Khoei, Phangnga Province (PN). Aerial part extract and root extract both had six dominant peaks (Figure 3). The same dominant peaks from chemical profiles there were not the same retention times.

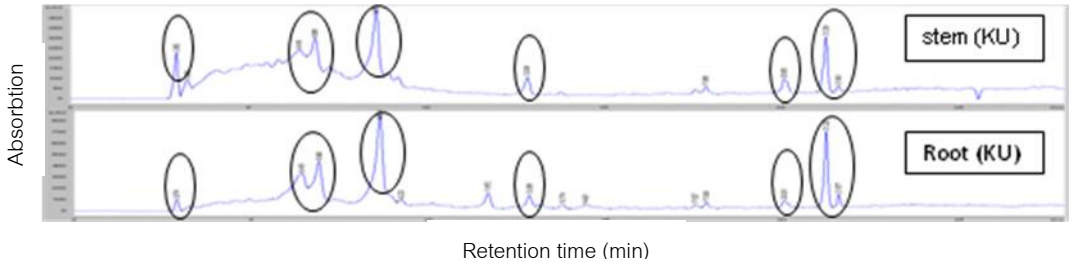

Figure 1 Comparison chemical profile between stem extract and root extract from KU

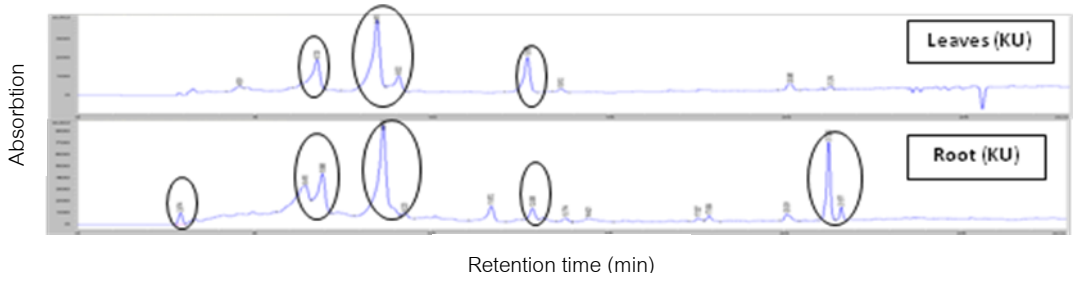

Figure 2 Comparison chemical profile between leaves extract and root extract from KU

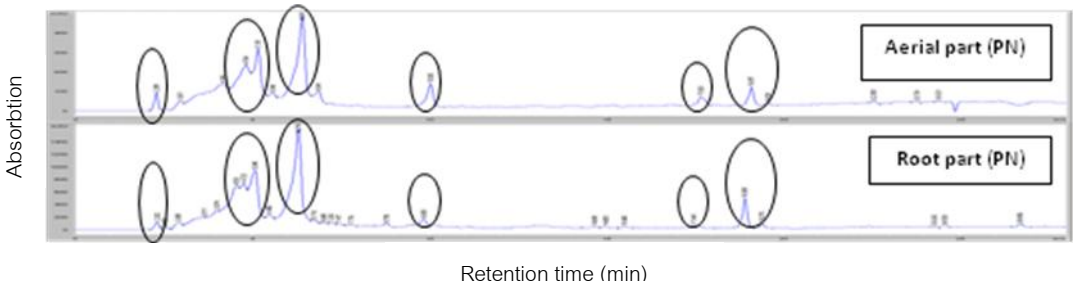

Figure 3 Comparison chemical profile between aerial part extract and root part extract from PN

Comparison of chemical profiles of the same plant parts collected from two habitats

For the two habitats, chemical profile of aerial part extract from PN had more peaks than leaves extract from KU, aerial part extract (PN) gave six dominant peaks and leaves extract (KU) showed four dominants peaks. The extract of root from KU presented eight dominant peaks for more than the extract of root from PN presented seven dominant peaks (Figure 4-5). The chemical profile of aerial part extracts and root extracts from Kasetsart University and Ao Khoei, Phangnga Province are different. Moreover, chemical profiles from HPLC analysis showed more differences between these two habitats. For this result, it might be the environment influence factor. Hence, future evidence such as cytology, anatomy or molecular evidence etc. For clearity, both chromatogram and UV spectra were used to determine the probability of identifying and comparing a chemical in each sample and isolating some remarkable compounds.

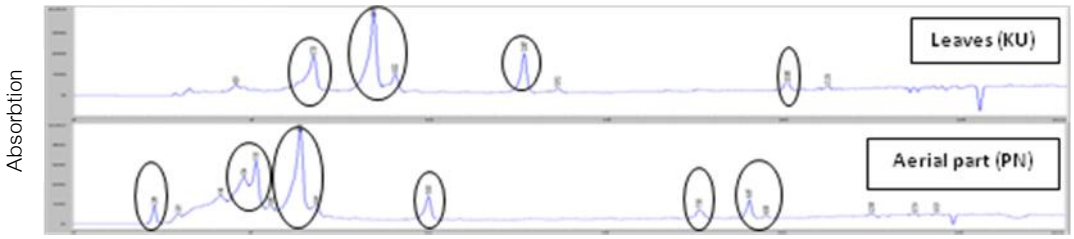

Figure 4 Comparison chemical profile of aerial part extract of *P. angulata* between KU and PN

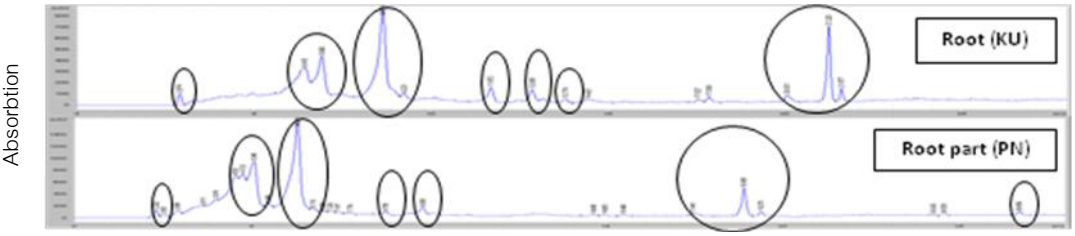

Figure 5 Comparison chemical profile of root extract of *P. angulata* between KU and PN

CONCLUSION

The morphology of *P. angulata* from two habitats, Kasetsart University and Ao Khoei, Phangnga Province were different. The chemical profiles using HPLC analysis in the same parts of the plants collected from two habitats were also different. And the phytochemical analysis of lipophilic extracts by TLC from aerial parts and underground parts of *P. angulata* from the two habitats showed the presence of alkaloids, terpenoids, steroids, coumarins and the general organic substances.

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