A comparison of the chemical compositions of herbal substitutes, *Panax ginseng*, *Codonopsis pilosula,* and *Panax notoginseng*, based on traditional Chinese medicine theory

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ABSTRACT

The chemical compositions of three herbal substitutes based on traditional Chinese medicine theory, namely *Panax ginseng*, *Codonopsis pilosula*, and *Panax notoginseng*, were examined and compared. From the preliminary screening of phytochemicals, all the three herbs contained terpenoid, flavonoid, saponin, steroid, tannin, alkaloid, and coumarin except for anthraquinone. Subjected to chromatographic fingerprinting using a mixture of cyclohexane/acetone/ethyl acetate/formic acid (20:8:5:0.3, v/v/v/v) as a mobile phase, all the extracts yielded a chromatogram with a band having a hR_f of 61 under UV366. Furthermore, upon visualization with 10% H_2SO_4/ethanol, three distinct bands with the hR_fs of 7, 72, and 81 were observed, while a band with the hR_f of 23 was only found in *P. ginseng* and *P. notoginseng*. These two findings suggested that *P. ginseng*, *C. pilosula*, and *P. notoginseng* shared a same set of chemical ingredients, with steroid saponins such as ginsenosides Rb1, Re, and Rg1 being the predominant kind. These results were corresponded to the therapeutic beliefs of traditional Chinese medicine, which stated that the three herbs could restore health and eradicate illness-causing agents while nourishing Yuan Qi and balancing the spleen, lungs, heart, kidneys, and blood. As a result, it suggested that *P. notoginseng*, *C. pilosula*, and *P. ginseng* might be utilized as herbal alternatives in clinical settings. Further study on the TLC fingerprint of the three plants using standard compounds as chemical markers is required to confirm that the same chemicals are present in all the three herbal extracts

Keywords: *Panax ginseng, Codonopsis pilosula, Panax notoginseng,* Herbal substitutes, Chemical compositions

INTRODUCTION

Panax ginseng, popularly called ginseng, is a costly medicinal herb that has been used for ages to promote blood circulation, maintain body equilibrium, and nourish the body.[1] In traditional Chinese medicine (TCM) formulations, *Codonopsis pilosula*, which likewise possesses strong body tonic properties, is commonly substituted for *P. ginseng* due to its lower cost. Additionally, one of the herbal alternatives to ginseng is *P. notoginseng*, which is a member of the same scientific genus and is usually used as a substitution for much more expensive *P. ginseng*.[2,3] These medicinal plants are herbal alternatives that are found in several TCM formulations, including Shen Fu Tang, Du Shen Tang, and Sheng Mai Yin.[4] According to a literature search, no comparative study on the chemical contents of these three herbal alternatives has been published. Thus, their chemical components were examined and compared using preliminary phytochemical screening and TLC fingerprinting methods to support the potential substitution between these plants based on the principle of traditional Chinese medicine.

METHODS

***General experimental procedures*:** All reagents used in this study are of analytical reagent grade except for technical grade solvents (dichloromethane and methanol) which were distilled *in vacuo* before being employed in the preliminary phytochemical screening. TLC was carried out using silica gel 60 F254 (Merck, Germany). The TLC Visualizer 2 operating with winCATS version 1.4.6.2002 (CAMAG, Switzerland) was utilized in this investigation along with the Linomat 5 TLC sampler (CAMAG, Switzerland), 100-mL microsyringe (Hamilton, Switzerland), and twin trough TLC chamber (20 x 10 cm) (CAMAG, Switzerland).

***Herbal materials*:** *P. ginseng*, *C. pilosula*, and *P. notoginseng* dried roots (**Figure 1.**) authenticated by a TCM doctor at V.P. Pharmacy Co., Ltd. in Bangkok, Thailand were supplied by the company. Following a thorough cleaning with water, they were placed in a hot air oven set to 50 °C for 5 hours to dry. The dried samples were then ground into powder. The resulting powder was passed through a sieve no. 80 and kept in a desiccator.

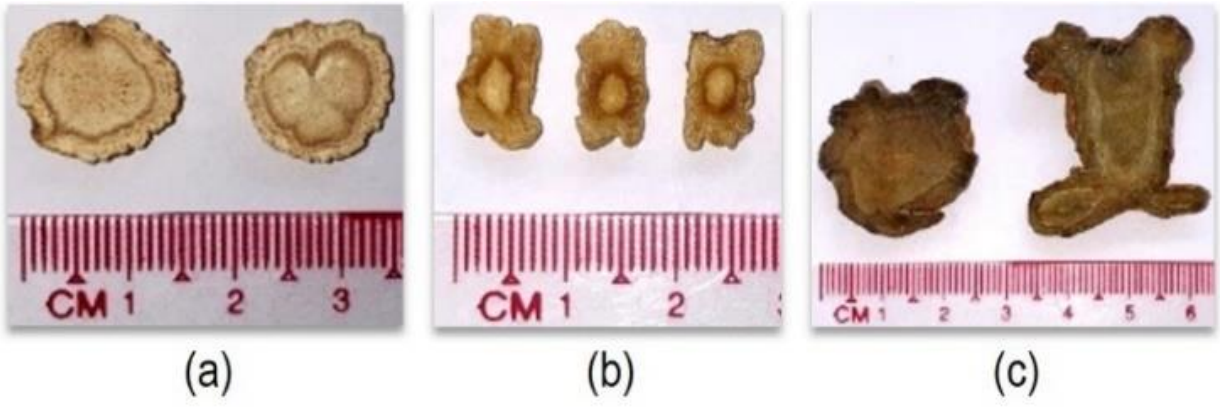

Figure 1. The physical appearance of *P. ginseng* (a), *C. pilosula* (b), and *P. notoginseng* (c) roots

***Phytochemical screening*:** Screening of the three herbs for various phytochemicals including anthraquinone, terpenoid, flavonoid, saponin, steroid, tannin, alkaloid, and coumarin constituents were carried out using standard methods[5-7] as summarized in **Table 1.**

TLC fingerprinting: One gram of each powdered sample was extracted with 10-mL methanol using an ultrasonicator for 10 min. After passing the mixture through Whatman filter paper no. 1, the obtained filtrate was dried *in vacuo*. Next, 1 mL of methanol was added to the extract material to dissolve it. Onto a TLC plate, 5 µL of each sample solution was spotted as a band of 8 mm. The plate development at room temperature was done using a TLC chamber pre-saturated with 20 mL of a mixture of cyclohexane/acetone/ethyl acetate/formic acid (20:8:5:0.3, v/v/v/v) for 20 min. The distance of the chromatogram run was 80 mm. After complete development, the plate was removed and left to dry in a fume hood. The developed plate was photographed under UV254 and UV366 as well as after staining with 10% H_2SO_4/ethanol and subsequent heating. The distance traveled by each component was measured, and their hR_f values were then calculated.

Table 1. Preliminary phytochemical tests used in this study

Phytochemicals	Test	Observation
Anthraquinone (Borntrager's test)	2 mL extract + 2 mL NH_4OH	Pink to red coloration in the ammoniacal layer
Terpenoid (Salkowski's test)	2 mL extract + 2 mL $CHCl_3$ + 1 mL conc. H_2SO_4	Reddish brown ring of the interface
Flavonoid (Shinoda's test)	2 mL extract + a few fragments of Mg ribbon + 1 mL conc. HCl	Pink to red coloration
Saponin (Froth test)	2 mL extract + 2 mL water + vigorously shaking	Persistent foam
Steroid (Liebermann-Burchard test)	2 mL extract + 2 mL acetic anhydride + 1 mL conc. H_2SO_4	Reddish brown ring at the junction
Tannin (Braymer's test)	2 mL extract + few drops of 5% $FeCl_3$	Blue-green coloration
Alkaloid (Mayer's test)	2 mL extract + few drops of Mayer's reagent	Creamy white precipitate
Coumarin (NaOH paper test)	a test tube containing moistened extract is covered with NaOH-treated filter paper + heating	Yellow fluorescence under UV366

RESULTS and DISCUSSION

***Phytochemical screening*:** The phytochemical screening displayed the presence of terpenoid, flavonoid, saponin, steroid, tannin, alkaloid, and coumarin in the extracts of *P. ginseng*, *C. pilosula*, and *P. notoginseng,* whereas anthraquinone was not observed (**Table 2**). In addition, a reddish brown ring of the interface was found from the three extracts in Salkowski's test indicating the presence of terpenoids (**Figure 2**). It was previously reported that dammarane-type triterpenoids were predominant bioactive components in ginseng.[1,8,9] This was in agreement with the results of the froth test and Liebermann-Burchard test which were positive in the three extracts (**Figure 2**), supporting that ginseng steroid saponins called ginsenosides found in *P. ginseng*, *C. pilosula*, and *P. notoginseng* were present.[1,9] The ginsenosides were responsible for the cardiac therapeutic effects of *P. ginseng* and *P. notoginseng* via the same molecular mechanism triggered by cardiac glycosides[10]; in terms of TCM theory, these benefits resulted from herbal medicines entering the heart and blood circulation, which further nourished the cardiovascular system.

Table 2. Phytochemical analysis results of the three herbal substitutes

Phytochemicals	*P. ginseng*	*C. pilosula*	*P. notoginseng*
1) Anthraquinone	-	-	-
2) Terpenoid	+	+	+
3) Flavonoid	+	+	+
4) Saponin	+	+	+
5) Steroid	+	+	+
6) Tannin	+	+	+
7) Alkaloid	+	+	+
8) Coumarin	+	+	+

+ = presence, - = absence

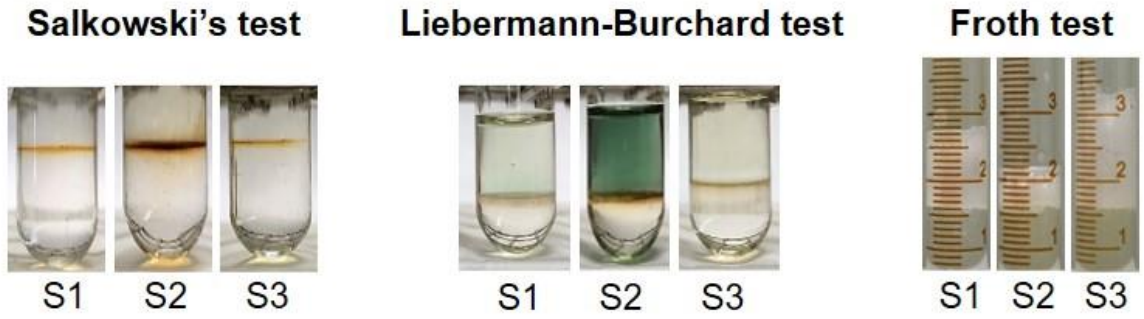

Figure 2. Some phytochemical analysis results of *P. ginseng* (S1), *C. pilosula* (S2), and *P. notoginseng* (S3) roots

TLC fingerprint: In the TLC fingerprinting of the dried root extracts from *P. ginseng*, *C. pilosula*, and *P. notoginseng*, their TLC chromatograms were obtained by the use of cyclohexane/acetone/ethyl acetate/formic acid (20:8:5:0.3, v/v/v/v) presented in **Figure 3.** Upon detection under UV254, UV366, and after visualization with 10% H_2SO_4/ethanol solution and subsequent heating, the TLC chromatograms of the three extracts displayed four different spots having hR_fs of 7, 61, 72, and 81. The obvious band at the hR_f of 61 was observed under UV366 (**Figure 3b, Table 3**), while the other 3 bands were detected after treating with 10% H_2SO_4/ethanol. The presence of these four bands in all the extracts indicated that they have the same chemical constituents. Interestingly, only *P. ginseng* and *P. notoginseng* gave the deep purple band at the hR_f of 23 (**Figure 3c**). It was reported that the same bioactive compounds found in *P. ginseng*, *C. pilosula*, and *P. notoginseng* belonged to steroid saponins called ginsenosides such as ginsenoside Rb1, Re, and Rg1, all of which possessed benefits to the immune, cardiovascular, and central nervous systems.[1,11] These benefits were corresponded to their medicinal properties based on TCM theory that help nourish Yaun Wi, balance the spleen, lungs, heart, and kidneys, and tonify Qi and blood, for example.[1] Both *P. ginseng* and *P. notoginseng* also contained other ginsenoside derivatives such as ginsenoside Ra1, Rb1, Rh2, Re, Rf, Rg1, Rg2, R1, R2, R3, R4, R5, and R6.[1]

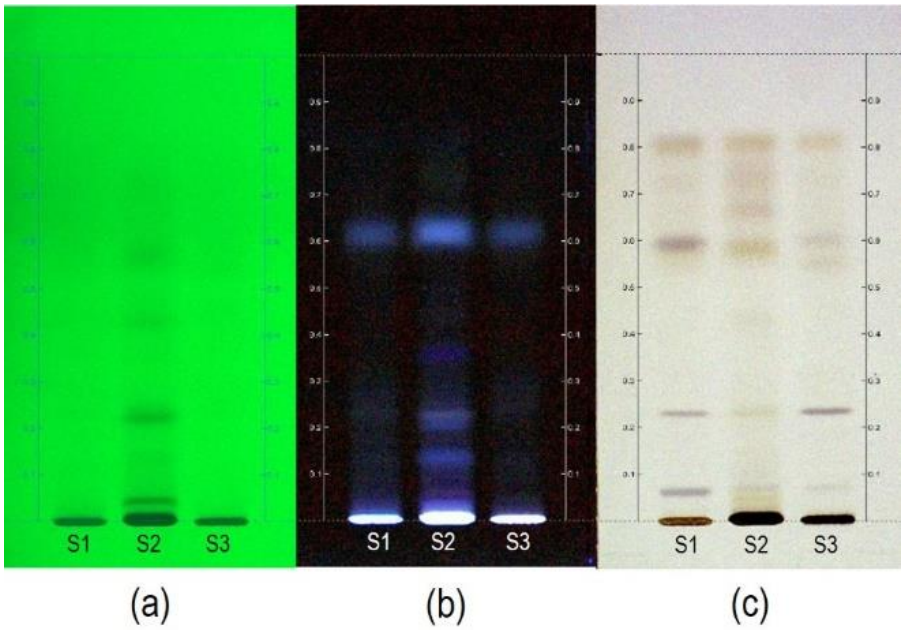

Figure 3. The TLC chromatograms of *P. ginseng* (S1), *C. pilosula* (S2), and *P. notoginseng* (S3) extracts observed under UV254 (a), UV366 (b), and after staining with 10% H_2SO_4/ethanol (c)

CONCLUSION

The phytochemicals of *P. ginseng*, *C. pilosula*, and *P. notoginseng* were determined and compared utilizing preliminary phytochemical screening and TLC fingerprinting. The phytochemical screening test indicated that they contained the same group of chemical ingredients including terpenoid, flavonoid, saponin, steroid, tannin, alkaloid, and coumarin except for anthraquinone. The TLC fingerprinting study of their extracts showed the presence of four different spots in the TLC chromatogram with hR_fs of 7, 72, and 81 being observed after visualization with 10% H_2SO_4/ethanol together with the hR_f of 61 being detected under UV366. These two findings suggested that *P. ginseng*, *C. pilosula*, and *P. notoginseng* shared the same set of chemical ingredients. These results corresponded to the therapeutic beliefs of traditional Chinese medicine, which stated that the three herbs could restore health and eradicate illness-causing agents while nourishing Yuan Qi and balancing the spleen, lungs, heart, kidneys, and blood. As a result, it suggested that *P. notoginseng*, *C. pilosula*, and *P. ginseng* might be utilized as herbal alternatives in clinical settings. Further study on the TLC fingerprint of the three plants using standard compounds as chemical markers is required to confirm that the same chemicals are present in all three herbal extracts.

Table 3. The hRf values of chemical constituents in the three herbal extracts

Plant extract	Spot no.	hRf	Color		
			UV254	UV366	10% H_2SO_4/ethanol + heating
S1: *P. ginseng*	1	7	-	-	Deep purple
	2	23	-	-	Deep purple
	3	43	-	-	-
	4	59	-	-	Deep purple
	5	61	-	Dark blue	-
	6	72	-	-	Brown
	7	81	-	-	Brown
S2: *C. pilosula*	1	4	Weak quenching	Light blue	Brown
	2	7	-	-	Brown
	3	12	Quenching	Light blue	-
	4	23	-	Light blue	Brown
	5	35	-	Light blue	-
	6	43	Quenching	-	Brown
	7	59	Quenching	-	Brown
	8	61	-	Dark blue	-
	9	66	-	-	Brown
	10	72	-	-	Brown
	11	81	-	-	Brown
S3: *P. notoginseng*	1	7	-	-	Deep purple
	2	23	-	-	Deep purple
	3	43	-	-	-
	4	54	-	-	Deep purple
	5	59	-	-	Deep purple
	6	61	-	Light blue	-
	7	72	-	-	Brown
	8	81	-	-	Brown

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