

The Determination of Total Alkaloid, Polyphenol, Flavonoid and Saponin Contents of Pogang gan (*Curcuma sp.*)

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Abstract

Pogang gan (*Curcuma sp.*, Zingiberaceae family) is medicinal herb and threatened and it is usually used as a tonic. We successfully micropropagated and cultivated this species. This study determined the total alkaloid, polyphenol, flavonoid and saponin contents of its growth time in order to determine harvest time. The highest level of total alkaloid content is roots after 11 months cultivation (0.272 mg/g), total polyphenol content is rhizomes after 11 months (1.71 mg GAE/g), total flavonoid content is rhizomes after 11 months (13.89 mg QE/g) and total saponin content is tubers and roots after 11 months (5.26% and 4.78%). The total content of alkaloid and flavonoid of pogang gan is higher than *Curcuma zedoaria*. The total saponin content of natural pogang gan is equal to 39.5% of 6-year old Vietnamese ginseng and the saponin content in rhizomes and tubers of 11-month-planted Pogang gan is from 55.5% to 67.8%, compared with natural Pogang gan.

Keywords: pogang gan, *Curcuma sp.*, alkaloid, polyphenol, flavonoid, saponin

1. Introduction

The medicinal herb **Pogang gan** (*Curcuma sp.*, Zingiberaceae family) has the narrow habit and low reserve, but it is usually used as a tonic. According to Bana ethnic, Pogang gan is as considered a medicine for the human. We successfully have propagated this species (Van Tan, 2016) but we have not determined the science name of Pogang gan's species yet because its inflorescence has been found. There are a lot of points in genus *Curcuma* among *Curcuma vitellina*, *C. singularis* and pogang gan via DNA analysis (Van Tan & Vinh, 2015).

Alkaloid, polyphenol, flavonoid and saponin have high pharmaceutical value and are rich of antioxidant, which are able to against free radicals and reduce blood fat (Drug Enforcement Administration (DEA), 2010). There for scientists are screening medicinal herbs which contain the mentioned matter to use them as medicine. Pogang gan (*Curcuma sp.*) contains higher contents of polyphenol, alkaloid, saponin than other species in the *Curcuma* genus, (good at heart operation and circulatory system and improves the people's health (Van Tan & Vinh, 2015)). However, we need to specify the content of the mentioned matter in rhizome, tuber and small root during the growing time in order to determine harvest time. Among the species of the *Curcuma* genus, white turmeric (*Curcuma zedoaria* Rosc.) is planted popularly in Vietnam and has a great value and contains high content of alkaloid, polyphenol and flavonoid (Dutta, 2015).

Vietnamese ginseng (*Panax vietnamensis* Ha & Grushv.) is the medicine which has a very high value, high saponin content and many other matters of saponin (Duc et al., 1993; Nguyen et al., 1995) that has higher value than other ginsengs (Report of Ministry of Health of Vietnam, 1978).

Total alkaloid, polyphenol, flavonoid and saponin contents are determined by contents in the underground parts of pogang gan and the proper harvest time of Pogang gan in order to evaluate the value of natural pogang gan and planted pogang gan, combined with white turmeric and vietnamese ginseng. This is the foundation to preserve and develop pogang gan.

2. Materials and Research Method

2.1 Materials Used in the Research

With regards to pogang gan, we used the parts: rhizomes, tubers and small roots which are collected from 2 places: Kon Bong 2 village, Dak Rong commune, Kbang distric, Gia Lai province (nature) and Chu Yang Shin

National garden, Krong Bong distric, DakLak Province (planted). The plants were harvested after 6 months and 11 months of the growth. Pogang gan was cultivated after 10 months the leaves and stems have faded only underground (rhizomes, tubers and roots) is alive (dormancy).

For white turmeric (*Curcuma zedoaria* Rosc.), we used rhizomes and tubers parts which was cultivated in DakLak province after 11 months.

For vietnamese ginseng (*Panax vietnamensis* Ha & Grushv.), rhizomes of plants which were cultivated in Dak Glei district, Kon Tum province after 6 years are used for this research.

All samples were dried up by solar energy, to reach the humidity 12%

2.2 Research Method

Sample-signs:

Ta0: rhizomes and tubers of white turmeric (*Curcuma zedoaria* Rosc.);

TaN: rhizomes and tubers of natural pogang gan (*Curcuma sp.*), after buds sprouting 11 months;

Ta1: rhizomes and tubers of pogang gan after 11-month-cultivated; at that time the aerial stems and leaves faded but root system is alive

Ta2: rhizomes of cultivated pogang gan after 6 months cultivated; at that time the trees have formated rhizomes and tubers;

Ta3: tubers of pogang gan after 11 months cultivated;

Ta4: tubers pogang gan after 6 months cultivated;

Ta5: small roots of pogang gan after 11 months cultivated;

SNL: rhizomes of 6 years old vietnamese gingseng (*Panax vietnamensis*);

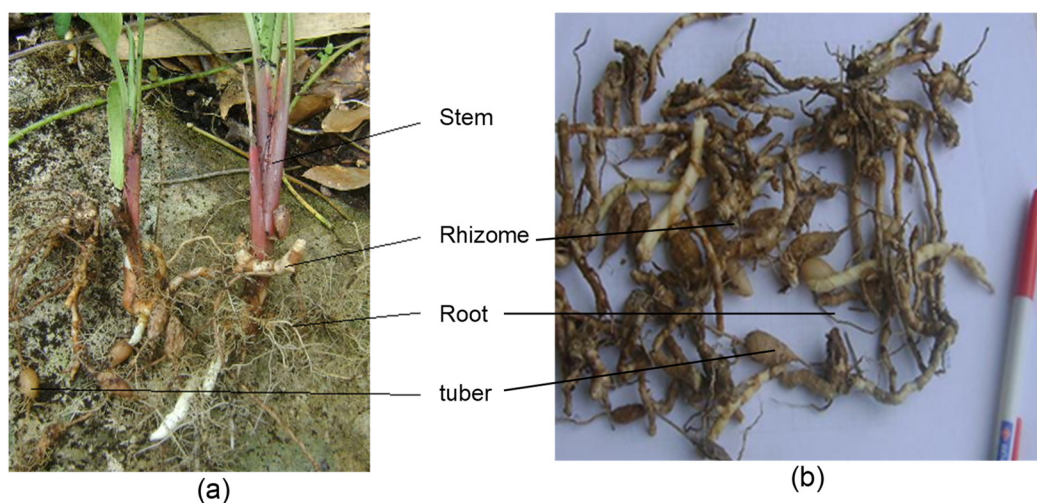


Figure 1. pogang gan: (a) trees of pogang gan; (b) a parts used (freshing)

In order to evaluate the contents of alkaloid, polyphenol, flavonoid and saponin, after drying up by solar energy, samples were desiccated at 105⁰C in 4 hours. All test analysis were repeated 5 times.

2.2.1 Determine Total Alkaloid Content

Determine total alkaloids by method of Shamsa, Monsef, Ghamooshi, and Verdian-rizi (2008).

Solution Preparation

Bromocresol green (BCG) solution were prepared by heating 69.8 mg bromocresol green with 3 ml NaOH 2N and 5 ml distilled water until completely dissolved. The solution was diluted to 1000 ml with distilled water. Phosphate buffer solution (pH 4.7) was prepared by adjusting the pH of sodium phosphate 2M (71.6 g Na₂HPO₄ in 1000 ml distilled water) to 4.7 with citric acid (42.02 g citric acid in 1000 ml). Atropine standard solution was made by dissolving 1 mg pure atropine (Sigma Chemical, USA) in 10 ml distilled water.

Preparation of Standard Curve

Accurately measure aliquots (0.4, 0.6, 0.8, 1.0 and 1.2 ml) of atropine standard solution and transfer each to different separated funnels. Then, add 5 ml pH phosphate buffer solution and 5 ml BCG solution and shake the mixture with 1, 2, 3 and 4 ml of chloroform. The extracts were collected in a 10 ml volumetric flask and then diluted to adjust the volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm blank prepared as above but without atropine.

Extraction

The plant materials (5 g) were ground and extracted with methanol for 24 h in a continuous extraction apparatus at 26°C. The extract was filtered and methanol was a rotary evaporator under vacuum at temperature of 45°C to dry. A part of this residue was dissolved in HCl 2N and then filtered. Once ml this solution was transferred to a separate funnel and washed with 10 ml chloroform (3 times). The pH of this solution was adjusted to be neutral with NaOH 0.1 N. Then 5 ml of BCG solution and 5 ml phosphate buffer were added to this solution. The mixture was shaken and the complex formed was extracted with 1, 2, 3, 4 ml chloroform by vigorous shaking. The extracts were collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm.

2.2.2 Determine total Polyphenol content

The total polyphenol content in the extracts was estimated using Folin-Ciocalteu method (Ahmed, Howladar, Mohamed, & Al-Robai, 2016; McDonald, Prenzler, Antolovich, & Robards, 2001). 1 ml galic acid solution in ethanol contents: 0.02, 0.04, 0.06 and 0.08 mg/ml added 5 ml Folin–Ciocalteu reagent, then add 4 ml sodium carbonate (75 g/l) was added 4.9 ml of distilled water. The mixture was then incubated at room temperature for 90 minutes. Absorbance was taken at 765 nm and total polyphenol content was expressed as mg galic acid equivalents per gram of dry samples (mg GEA/g).

2.2.3 Determine total Flavonoid content

Determination flavonoid content was by conducted using the method presented by Jia, Tang, and Wu (1999). The Flavonoid in the samples will be yellow complex formulation with $AlCl_3$ solution. The color intensity is directly proportional to the amount of flavonoid content, which is determined at 510 nm wavelength.

Take 0.5 ml of quercetin solution in methanol with 0.025, 0.050, 0.075, 0.10 and 0.15 mg/ml for 2.5 ml of distilled water, adding 0.15 ml $NaNO_2$ 5%, Hold for 5 minutes, then add 0.3 mL of $AlCl_3$ 10%, hold for 5 minutes, continue to add 1 ml NaOH 1M and 0.55 ml of distilled water. The absorbance of the solution was determined at 510 nm wavelength, establishing the calibration curve and the regression equation between the quercetin concentration and the absorbance.

2.2.4 Determine Total Saponin Content

The determination total saponin content was based on the method of Nguyen et al. (1995). After being extracted 3 times with ethanol, the samples are dried by a rotary evaporator at 55°C. Then extraction is dissolved with water and extracted by chloroform to remove lipids and is final extracted by n-butanol. This n-butanol extract was evaporated on a rotary evaporator until constant mass. The n-butanol mass is the mass of total saponin.

Determine to identify compounds of saponin by thin layer chromatography (TLC) with dynamic solvent butanol: ethanol: ammoniac (6:3:3) and observe sample at 365 nm wavelength.

The figures are evaluated the average, STDEV and graphed by Excel.

3. Results and Discussion

3.1 Total Alkaloid Content

Table 1. shows that total alkaloid content of Pogang gan's rhizome and small root is higher than white turmeric, excepts tubers and rhizome of 6-month-planted. Total alkaloid content between natural Pogang gan (collected from Kon Bong 2 village, Dak Rong commune, Kbang district, Gia Lai province) and 11-month-cultivated Pogang gan are not significantly different. The highest content of total alkaloid is found in the small roots (0.273 mg/g) but root only contribute 5% productivity. A.R. Srividya et al. found out alkaloid in *C. zedoaria* planted in India (Srividya, Dhanabal, Ajit Kumar, Sathish Kumar, & Vishnuvarthan, 2012). Moreover, Abdelazim Ali Ahmed et al. not only found out alkaloid in *Achyranthes aspera* in Albaha area (Saudi Arabia) (Ahmed, Howladar, Mohamed, & Al-Robai, 2016), but also specified that the alkaloid content with very high level, about 9.2mg/g.

Table 1. Total alkaloid content

Kind of sample	total alkaloid (mg/g)
Ta0	0.197 ± 0.008
TaN	0.251 ± 0.006
Ta1	0.225 ± 0.005
Ta2	0.165 ± 0.005
Ta3	0.194 ± 0.005
Ta4	0.182 ± 0.004
Ta5	0.273 ± 0.004

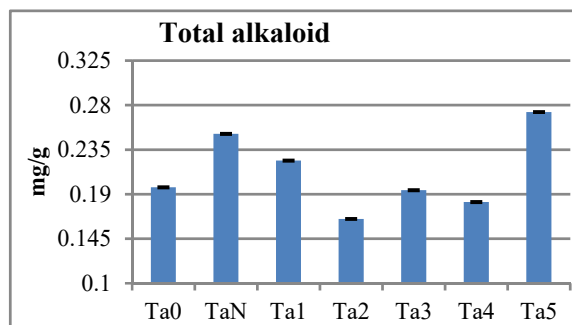


Figure 2. total alkaloid (mg/g)

3.2 Total Polyphenol Content

Table 2. Total polyphenol content

Kind of sample	Total polyphenol (mg GAE/g)
Ta0	1.81 ± 0.026
TaN	2.35 ± 0.054
Ta1	1.71 ± 0.043
Ta2	1.40 ± 0.064
Ta3	0.55 ± 0.052
Ta4	0.43 ± 0.064
Ta5	1.15 ± 0.068

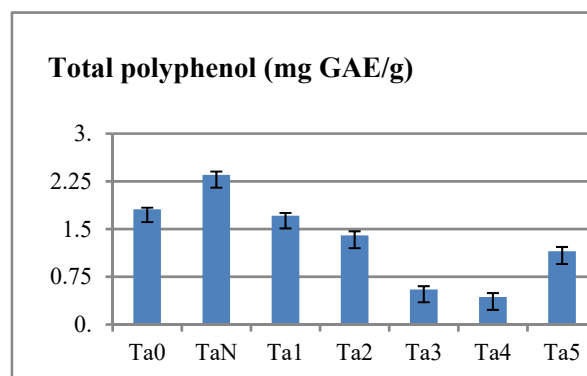


Figure 3. total polyphenol (mg GAE/g)

Note: GAE: gallic acid equivalent

Table 2. shows that total polyphenol content in rhizomes and tubers of natural Pogang gan is the highest (2.35mg GAE/g), higher than white turmeric (1.81 mg GAE/g). Total polyphenol content in rhizomes of 11-month-cultivated Pogang gan is approximated to white turmeric (1.71/1.81mg GAE/g). Polyphenol content accumulated from time to time is shown clearly. This accumulation in the 6-month-cultivated Pogang gan is equal to 81% of 11-month-cultivated Pogang gan, and is equal to 59% of natural pogang gan. The polyphenol content is low in the roots and the lowest in tubers (approximated 0.5mg GAE/g). The total polyphenol content of the mentioned samples is significantly lower than the polyphenol content of white turmeric in India (34.45 mg GAE/g) (Srividya, Dhanabal, Ajit Kumar, Sathish Kumar, & Vishnuvarthan, 2012).

3.3 Total Flavonoid Content

Table 3. Total flavonoid content

Kind of sample	Total Flavonoid (mg QE/g)
Ta0	6.06 ± 0.07
TaN	14.48 ± 0.10
Ta1	13.90 ± 0.05
Ta2	8.23 ± 0.02
Ta3	0.62 ± 0.02
Ta4	0.28 ± 0.01
Ta5	9.11 ± 0.01

Note: QE: quercetin equivalent

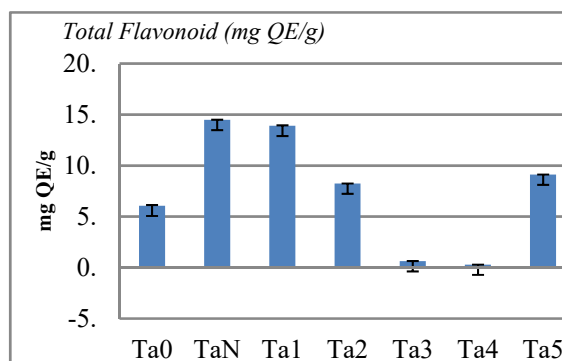


Figure 4. total flavonoid (mg QE/g)

The member of polyphenol group but flavonoid is the most important and the most used. Table 3, shows that flavonoid presents in all the pogang gan samples. The flavonoid content of pogang gan's rhizome is higher than flavonoid of rhizomes and tuber of white turmeric, but flavonoid content of tuber is low. After 11 months cultivated, the flavonoid content in rhizomes is approximated to that content in natural Pogang gan (13.49/14.45 mg QE/g) and is doubled higher in white turmeric (6.06 mg QE/g). The flavonoid content of pogang gan and white turmeric planted in Vietnam is lower than the flavonoid content of white turmeric cultivated in India (45.56 mg QE/g) (Srividya, Dhanabal, Ajit Kumar, Sathish Kumar, & Vishnuvarthan, 2012).

3.4 Total Saponin Content

Table 4. Total saponin content

Kind of sample	Total saponin content (%)
SNL	19.49 ± 0.062
TaN	7.75 ± 0.078
Ta1	4.32 ± 0.032
Ta2	0.75 ± 0.084
Ta3	5.26 ± 0.027
Ta4	2.95 ± 0.039
Ta5	4.78 ± 0.037

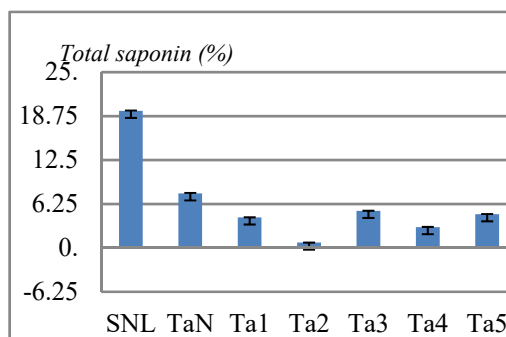


Figure 5. total saponin content (%)

The saponin in ginseng is ginsenosides, is good for nervous system, hormon, immune system, metabolism and accommodates body activities (Drug Enforcement Administration (DEA), 2010). All parts of pogang gan contain saponin. However, the highest content is in the rhizomes and tubers of 11-month natural pogang gan (7.75%), equal to 39.5% of 6 year old vietnamese ginseng (19.49%). The saponin content of cultivation highest is in tuber of 11 months (5.26%). The saponin content of the natural pogang gan is higher than the cultivated it. The saponin content of tuber is higher than rhizome, but quantity of tuber contribute about 20% harvest. The saponin content of rhizomes and tubers of 11-month-cultivated pogang gan is between 55.5% and 67.8% compared with natural Pogang gan. The accumulation of saponin over time is very clear, affter 11-months is higher than 6 months.

3.5 The Compounds in Saponin of Pogang Gan

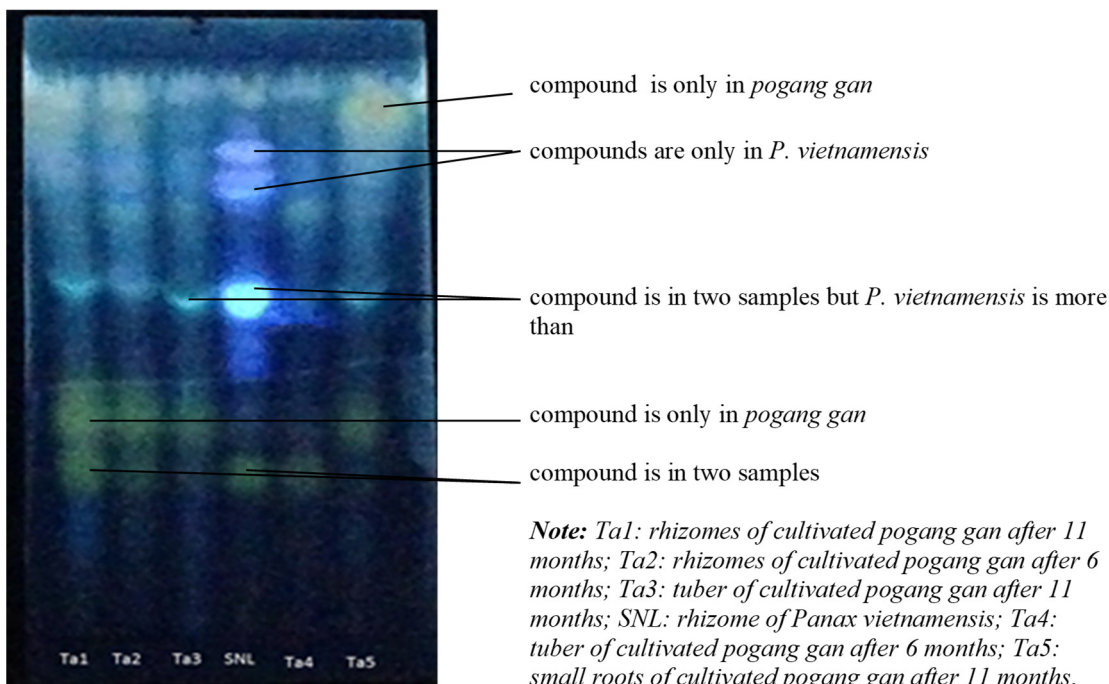


Figure 6. The compounds in saponin by thin layer chromatography

4. Conclusion

All parts of pogang gan (*Curcuma sp.*) contain alkaloid, polyphenol, flavonoid. The contents of these compounds are higher than white turmeric. Total saponin content of natural Pogang gan is equal 39.5% to vietnamese ginseng. And there are some matters which are similar to vietnamese ginseng. After planting 11 months, total alkaloid content is accumulated on rhizomes and tubers. The highest content of alkaloid is found in rhizome. Total polyphenol and flavonoid content are accumulated in rhizomes. Total saponin content is accumulated on rhizome and tuber.

We have found that pogang gan harvested after 11 months has contained more alkaloid, polyphenol and flavonoid than that in white turmeric, which is favorable to Vietnamese farmer to reduce harvesting time from (normal duration for example several years) to 11 months.

High saponin content, approximately 40% vietnamensis ginseng. Shows that pogang gan is a valuable tonic.

After cultivation pogang gan 11 months productivity 250 gam/tree, among of them rhizome contribute 90%, tuber 5% and root 5%. The fresh/dry ratio is 7.5. This produces a valuable source of medicinal herb.

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