Original Research Article

Panax sp. in Tuyen Quang, North Vietnam – A Potential Plant for Poverty Reduction

4

1

2

3

5 6

7

8

9

10

11

12 13

14 15

16

17

18

19

20

2122

ABSTRACT

Ginsengs are perennial forest herbs, belonging to genus *Panax* L. A species of ginseng was found in Tuyen Quang province, North Vietnam and named as Panax sp. - Tuyen Quang ginseng. Understanding the ecology, morphology, and saponin of Tuyen Quang ginseng becomes important for development, which can contribute to poverty reduction in the province. Field survey was conducted for ecology and samples were collected for morphology description and saponin analysis. The results indicates Tuyen Quang ginseng distributes in evergreen broadleaved forests in elevation of 980-1,200 m above sea level. It grows in forest with canopy cover of >50% and low vegetation cover of >80%. The soil must be acidic with pH of 4.5-5. Soil contains high ratio of sandy particles and is drainage. Morphology indicates some differences of Tuyen Quang ginseng with other described ginsengs in Vietnam. Saponin content of Tuyen Quang ginseng is 13.7%, which is much higher than that of *Panax stipulealatus* (7.1%) and *P. bipinnatifidus* (7.5%), two widely planted and marketed ginsengs in Vietnam. In addition, current price of Tuyen Quang ginseng is up to 2,600 US\$/1 kg. It is concluded that Tuyen Quang ginseng could be considered as a potential forest herb for poverty reduction. However, study on growing techniques should be conducted before practical application.

23

24

Keywords: Ethnic community; Panax sp.; poverty reduction; saponin; Tuyen Quang.

1. INTRODUCTION

26

- 27 The genus Panax L. belonging to family Araliaceae is called ginseng. Until recently, 19
- 28 Panax species and subspecies have been described worldwide, most of them grow in eastern
- 29 Asia [1]. Ginsengs have been widely used as traditional medicines [2-3]. The ginsenosides
- 30 (triterpene glycosides) are the main biologically-active compounds of *Panax* L, which was
- 31 first found 50 years ago. Until recently, more than 150 different ginsenosides have been
- isolated from different *Panax* species[4-5].
- 33 In Vietnam the first ginseng was found in 1973 and named as Panax vietnamensis Ha et
- Grushv. [6]. In 2003, a subspecies of P. vietnamensis was described and named as P.
- 35 vietnamensis var fuscidicus K. Komatsu, S. Zhu & S.Q. Cai. This subspecies has natural
- distribution in south part of Yunnan province, China and Laichau province, North Vietnam
- 37 [7-8]. Until recently, three ginseng species (P. vietnamensis Ha et Grushy, P.
- 38 stipulealatus Tsai & K.m. Feng, and P. bipinnatifidus Seem.) and two subspecies (P.
- 39 vietnamensis var fuscidicus and P. vietnamensis var. langbianensis) have been found in
- 40 Vietnam.
- 41 Tuyen Quang province locates in North Vietnam with diversity of vegetation types,
- 42 topography, and climate conditions. A ginseng (Panax sp.) was found to have natural
- distribution in narrow areas of Lam Binh and Na Hang districts of Tuyen Quang province, so
- 44 called "Tuyen Quang ginseng". It has been harvested from natural for health improvement
- 45 and marketing to generate income for local ethnic communities for decades. The objectives of
- 46 this study were (1) to describe ecological and morphological characteristics and (2) to
- analyze roots for saponin content of Tuyen Quang ginseng, which could be used to develop
- and manage this economically valuable ginseng sustainably.

49

50

51

2. MATERIALS AND METHOD

2.1 Materials

- 52 By interviewing local authorities and ethnic people, it was reported that Tuyen Quang
- 53 ginseng has natural distribution in Sinh Long commune, Na Hang district and Thuong Lam
- 54 commune, Lam Binh district of Tuyen Quang province (Fig. 1). Therefore, those two
- communes were selected for field survey.

57

2.2 Methods

- 58 2.2.1 Natural and Ecological Characteristics
- By interviewing local people, who have found and harvested Tuyen Quang ginseng, the areas
- of natural distribution became known. With their field guidance, it was easy for the survey
- 61 team to get the desired survey locations. In the field, if plant of Tuyen Quang ginseng was
- 62 found, natural and ecological characteristics were described including forest type, height of
- 63 forest canopy, vertical structure of forest canopy, forest canopy cover, and low vegetation
- layer. In addition, a soil pit of 1.2 m depth and 60 cm width was dug up for soil profile, and a
- soil sample was collected from 0-20 cm soil depth for analyzing in Lab.

66

- 67 2.2.2 Morphological and Anatomy Characteristics
- 68 Five fully-developed plants of Tuyen Quang ginseng were randomly selected for
- 69 morphological characteristics including leaves, stem, root, flower, fruit, and seed. Shape,
- 70 color, and dimension of each organ were described through observation naked eyes. In
- 71 addition, slices of stems and roots were also used for their structure by observing through
- microscope [9]. Slices of 40-60 µm was cut by using special equipment. The slice was then
- 73 colored by chrysanvioles (0.5%) and safranin (1%). It was then cleaned by purified water and
- observed through microscope.

75

- 2.2.3 Saponin Content
- 77 Total saponin was estimated by be weighing. Approximate one gram powder of the dried root
- 78 sample were weighed, 100 ml *n*-hexan were then added. The mixture was extracted in
- 79 Soxhlet extraction apparatus in six hours and filtered. The residue was continuously extracted
- 80 by Soxhlet extraction apparatus with 100 ml of 70% methanol for 6 hours. The combined
- 81 filtrate was concentrated under reduced pressure with a rotary evaporator to obtain extract.
- 82 The methanol extract was diluted in 30 ml of water and then fractionated with water saturated
- 83 *n*-butanol until no color was observed in n-butanol layer. The filtrated butanol extract was
- 84 then evaporated under reduced pressure to yield butanol extract. This extract was dissolved
- 85 into 10 ml of 70% ethanol then transferred into a porcelain beaker and evaporated solvent to

get extract. The obtained extract was dried in an oven at 105 °C until constant weight. Total saponin content (X) was calculated as: $X = [(b \times 100)/(m \times (100 - d))] \times 100$, where b is obtained saponin weight (g), d is moisture of root powder (%), and m is initial weight of root powder (g).

90

91

3. RESULTS

92 **3.1 Ecology**

- 93 Tuyen Quang ginseng naturally distributes in evergreen broadleaved forests of both disturbed
- and undisturbed forests. The height of forest canopy is taller than 15 m. The canopy structure
- 95 includes two or three layers with the shortest layer of 7-10 m tall and the difference between
- layers of 4-6 m. Tuyen Quang ginseng prefers to grow in forests with canopy cover of >50%.
- 97 The species composition of forest canopy, where Tuyen Quang ginseng grows, includes tree
- 98 species of Saurauia napaulensis DC., Callicarpa arborea Roxb., Alniphyllum eberhardtii
- 99 Guillaumin, Schefflera macrophylla (Dunn) R.Vig., Machilus chinensis (Benth.) Hemsl., and
- 100 Pavetta indica L. with density of 650-860 trees/ha. The vegetation layer on forest floor
- 101 includes plants of Asarum caudigerum Hance, Lophatherum gracile Brongn., Alpinia
- 102 chinensis (Retz.) Roscoe, Cyclosorus parasiticus (L.) Farw., and Polygonum chiensis L. with
- cover >80%. The litter layer on forest floor is thick and wet most time of the year.
- 104 Suitable climate conditions for Tuyen Quang ginseng include annual precipitation of 1,700-
- 1,900 mm, annual air humidity of >85%, annual air temperature of 23°C, minimum
- temperature of >5°C, and maximum temperature of <30°C. Tuyen Quang ginseng distributes
- on elevation zone of 980-1,200 m above sea level, growing well in mountain foots, flat areas,
- and well drainage soils.
- Soil profile indicates the litter layer of 4-5 cm, humus layer of 2-3 cm with dark color and
- humidity of 56-60%, and total soil depth of >100 cm. Tuyen Quang ginseng naturally grows
- in acidic soil with pH of 4.5-5.0, high humus soil of 6.5-9% (Table 1). Content of sand
- particles is high, indicating drainage soil. Nitrogen is not much required by Tuyen Quang
- ginseng as it can grow in low N content soil of 0.5%.

114

115

3.1 Morphology

- Tuyen Quang ginseng is a perennial plant, up to 60 cm tall at maturity (Fig. 2). Root lies
- 117 horizontally without root branching. Each root contains one aerial stem with leaves. In very
- rare case, it may contains 2-3 aerial stems.
- Root is brown to light yellow (Fig. 2) with numerous scars as results of dead aerial stems of
- each growing year. Scars arranges alternately in root. The head of root is big and global,
- while the end of root is much smaller and contains bunch of small roots for absorbing water
- and nutrient. Inner part of roots is light yellow or violet (Fig. 2). Total root length is 3.2-26.5
- cm and diameter is 1.2-3.3 cm.
- Aerial stem dies in winter and new stem starts from root head in spring. This creates scars in
- roots (Fig. 2). Aerial stem is 20-60 cm height and 0.3-0.8 cm diameter. It is green and/or light
- violet without hairs (Fig. 3). Each aerial stem contains 1-4 main leaves and each main leaf
- contains 4-6 sub-leaves (Fig. 3). There are tiny hairs covering both sides of sub-leaves (Fig.
- 128 3).
- Each aerial stem contains an inflorescence on top (Fig. 4), which is 15-25 cm length and
- contains 40-120 flowers. The inflorescence is 3-4.5 cm in diameter. When ripen, fruits
- become red. Seeds are white with a triangle shape. Flower season is May-July and season of
- ripen fruits is October-December.

134

3.3 Anatomy and Saponin

- 135 Aerial stem and root of a 6-year old plant of Tuyen Quang ginseng were collected for
- anatomy and saponin analysis. Results indicates aerial stem of Tuyen Quang Ginseng
- includes main parts as cutin layer in outermost, epidermis, xylem, phloem, and xylem ray
- 138 (Fig. 5). While, transverse section of root indicates main layer of epidermis, oxalate, phloem,
- 139 xylem, and xylem ray (Fig. 6).
- Total saponin content of Tuyen Quang ginseng (*Panax* sp.) is 13.7%, which is much higher
- than that of *P. stipuleanatus* and *P. bipinnatifidus* (Table 2). However, it is lower than *P.*
- vietnamensis var. fuscidiscus (21.9%) and P. vietnamensis (22.3%), two well-known ginsengs
- in Vietnam.

144

145

4. DISCUSSION

146 Forest herbs like ginseng can only survive and grow well under shades of other vegetation 147 [10-12]. Therefore, Tuyen Quang ginseng can only be found in evergreen broadleaved with 148 forest canopy cover of >50%, and lower vegetation cover of >80% in the present study. 149 Forest structure including more than two layers of trees, and lower vegetation [13] is an 150 important indicator for the existence of Tuyen Quang ginseng. Low cover of tree layer will 151 allow direct sunlight to forest floor, reducing soil moisture, litter layers, soil organic carbon 152 etc., which is not a favor condition for existence of Tuyen Quang ginseng [14]. 153 Soil with high ratio of sandy particles and high humus content (Table 1) indicates low 154 compact soil, which supports growth of roots in the soil. If soil is compact with low humus content, the soil is high water holding capacity with low soil air. Such kind of soil will never 155 156 well support growth of plants which have roots as main part of their body. Therefore, selecting suitable soil is important for growing Tuyen Quang ginseng. It must be high humus 157 158 content and drainage soil. 159 Comparing morphology among five ginsengs in Vietnam indicates several differences of Tuyen Quang ginseng with others (Table 3). The most different characteristic is number of 160 161 leaves per aerial stem, which contains 1-4 leaves per stem in Tuyen Quang ginseng compared 162 to 3-5 in others. While, no. flowers were also most numerous of up to 120 flower per 163 inflorescence. It seems that Tuyen Quang ginseng most look like P. vietnamensis. However, 164 there is a possibility of a new sub-species found in Tuyen Quang province. Therefore, further 165 study such as DNA analysis is required to identify where the Tuyen Quang ginseng (Panax 166 sp.) is a new sub-species of ginseng in Vietnam. 167 Anatomy of Tuyen Quang ginseng is similar to that of other ginseng and plants [9, 15], which 168 have been widely used as traditional medicines. Root shows clusters of oxalate, which is 169 known as containing high saponin; the main active chemical component of ginseng. 170 The saponin content of Tuyen Quang ginseng is much higher than that of P. stipulealatus 171 and P. bipinnatifidus (Table 2), which have been widely grown and marketed for poverty reduction in mountainous areas of Vietnam. In addition, current price of Tuyen Quang 172 173 ginseng ranges 1,000-2,600 US\$/ 1 kg, depending on size and age of roots [16]. Therefore, 174 growing Tuyen Quang ginseng could also contribute to poverty reduction in Tuyen Quang province. However, before practical application researches on planting Tuyen Quang ginseng 175

should be conducted extensively to issue the applicable growing guideline, ensuring the

quality/saponin content of planted ginseng compared to natural ones.

5. CONCLUSION AND RECOMMENDATION

179 Tuyen Quang ginseng -Panax sp. has natural distribution in Thuong Lam commune Lam 180 Binh district and Sinh Long commune, Na Hang district of Tuyen Quang province, North Vietnam. The species distributes in evergreen broadleaved forests on the elevation of 980-181 182 1,200 m above sea level. The species only distributes in forests with canopy cover >50% and cover of low vegetation layer of >80%. The soil must be acidic with pH of 4.5-5.0 and high 183 184 ratio of sandy particles, indicating drainage soil. 185 There is a possibility that Tuyen Quang ginseng is a new sub-species of ginseng because of 186 some differences of its morphological characteristics compared to other ginsengs in Vietnam. 187 However, detail analysis such as DNA application should be conducted for better conclusion. 188 Tuyen Quang ginseng has high saponin content (13.7%) and is a potential forest herb for 189 poverty reduction to ethnic communities in the province. Study on growing techniques must 190 be conducted, which is valuable for local communities. Selecting vegetation types for 191 growing Tuyen Quang ginseng is important, which must have high canopy cover of > 50% 192 and high cover of low vegetation layer of >80%.

193

178

194

196 **REFERENCES**

- 1. Pandey AK, Ali MA. Intraspecific variation in *Panax assamicus* Ban. Populations based on internal transcribed spacer (ITS) sequences of nrDNA. International Journal Biotechnology. 2012;11:30–38.
- 200 2. Shim SC, Chang SK, Hur CW, Kim CK. New polyacetylene compounds from *Panax* 201 *ginseng* C. A. Meyer. Bulletin of Korean Chemical Society. 1987; 8:272–275.
- 3. Kochkin DM, Kachala VV, Shashkov AS, Chizhov AO, Chirva VY, Nosov AM. Malonylginsenoside content of a cell-suspension culture of *Panax japonicus var*. repens. Phytochemistry. 2013; 93:18–26.
- 4. Qi LW, Wang CZ, Yuan CS. Ginsenosides from American ginseng: chemical and pharmacological diversity. Phytochemistry. 2011; 72:689–699.
- 5. Yoshizaki K, Murakami M, Fujino H, Yoshida N, Yahara S. New triterpenoid saponins from fruit specimens of *Panax japonicus* collected in Toyama prefecture and Hokkaido (2). Chemical and Pharmaceutical Bulletin. 2012; 60:728–735.
- 6. Dung HT, Grushvisky IV. A new species of the genus*Panax* L., Araliaceae in Vietnam:
 Panax vietnamensis Haet Grushv. Botany Journal Vietnam. 1985; 70:518–522.
- 7. Zhu S, Fushimi H, Qing CS, Biao CH, Komatsu K. A new variety of the genus Panax from
 Southern Yunnan, China and its nucleotide sequences of 18S ribosomal RNA gene and
 matK gene. Journal of Japanese Botany. 2003; 78: 86–94.
- 8. Phan KL, Le TS, Phan KL, Vo DD, Phan VT. Lai Chau ginseng *Panax vietnamensis var.* fuscidiscus K. Komatsu, S. Zhu & S.Q. Cai I. morphology, ecology, distribution and conservation status. Proceeding of the 2nd VAST-KAST Workshop on Biodiversity and Bio-active compounds.2013; pp. 65–73.
- 9. Liu EH, Qi LW, Li K, Chu C, Li P. Recent advances in quality control of traditional Chinese medicines. Combinatorial Chemistry & High Throughput Screening. 2010; 13:869–884.
- 10. Anderson RC, Fralish JS, Armstrong JE, Benjamin PK. The ecology and biology of Panax quinquefolium L. (Araliaceae) in Illinois. American Midland Naturalist.1993; 129:357–372.
- McKenzie D, Halpen CB, Nelson CR. Overstory influences on herb and shrub
 communities in mature forests of western Washington, U.S.A. Canadian Journal ofForest
 Research. 2000; 30:1655–1666.
- Pabst RJ, Spies T. Distribution of herbs and shrubs in relation to landform and canopy
 cover in riparian forests of coastal Oregon. Canadian Journal of Botany. 1997; 76:298–
 315.
- 231 13. Turner IM, Tan HTW, Chua KS. Relationships between herb layer and canopy composition in a tropical rain forest successional mosaic in Singapore. Journal ofTropical Ecology 1996; 12:843–851.
- 14. Beyfuss R. Growing ginseng fact sheet. USDA, Natural Resources Conservation Service
 401. 2000. http://hwwff.cce.cornell.edu/docs/GinFs.pdf(accessed: 1 May, 2018)
- 15. Tengku AS, Tengku M, Humera N, Ratni SJ, Khatijah H, Mohd RAR, Aishad A, Jean FredericFW. Chemical and pharmacognostical characterization of two Malaysian plants
 both known as Ajisamat. Revista Brasileira de Farmacognosia. 2013; 23:724–730.
- 16. Pham QT. Growing and developing Laichau ginseng. Scientific Report. Silviculture
 Research Institute, Vietnamese Academy of Forest Sciences. Hanoi. 2018.

Table 1. Physical and chemical characteristics (\pm SE) of soil samples

Research site	Density (g/cm ³)	Silt (< 0.002 mm; %)		am 2-0.02 ; %)	Sand (2-0.02 mm; %)	pН	Humus content (%)	N (%)	K ₂ O (%)
Thuong Lam, Lam Binh	1,135.8 ±27.0	12.7 ±0.9	33.0	±1.9	54.3 ±2.7	4.5 ±0.3	6.7 ±0.9	0.5 ±0.1	2.5 ±0.3
Sinh Long, Na Hang	1,156.5 ±8.5	4.3 ±0.0		34.9 ±1.4	60.8 ±1.4	4.9 ±0.3	8.9 ±1.1	0.6 ±0.0	3.2 ±0.3

Table 2. Saponin content of ginsengs in Vietnam

Species	Root source	Saponin content (%)	
Panax sp Tuyen Quang ginseng (study species)	Natural, Tuyen Quang province	13.7	
Panax stipulealatus Tsai & K.m. Feng	Growing in garden of local people in Laichau Province, five years old	7.1*	
Panax bipinnatifidus Seem.,	Growing in garden of local people in Laichau Province, five years old	7.5*	
Panax vietnamensis var fuscidicus K. Komatsu, S. Zhu & S.Q. Cai.	Growing in garden of local people in Laichau Province, five years old	21.9*	
Panax vietnamensis Ha et Grushv	Natural, central Vietnam	22.3*	

245 *cited from Pham et al. [16].

Table 3. Morphological characteristics of five ginsengs in Vietnam.

	Species					
	P. stipuleanatus	P. vietnamensis	P. vietnamensis var. fuscidiscus	P. notogingseng	Panax sp. (Tuyen Quang ginseng)	
Root distribution	Horizontal	Horizontal	Horizontal	Vertical	Horizontal	
Root scar	In line	Alternate	Alternate	Random	Alternate	
Color of inner part of root	White green	Yellow or light violet	Yellow or light violet	Yellow or light violet	Yellow or light violet	
Aerial stem	Violet or green	Violet	Violet	Violet	Green or light violet	
Leaves per stem	3	3-5	3-5	4	1-4	
No. flowers	60-80	50-120	40-120	40-120	40-120	
Flower color	White green	White green	White green	White green	White green	
Stamen	1	1-2	1-2	2-3	1-2	
Pistil	1-2	1-2	1-3	2-3	1-2	
Color of ripen fruit	Red	Red with black dots on top	Red with black dots on top	Red	Red	
Shape of fruit	Kidney shape	Kidney shape	Kidney shape	Half global or triangle	Kidney shape or half global	
Seeds per fruit	1-2	1-2	1-3	2-3	1-2	
Shape of seed	Kidney shape	Kidney shape	Kidney shape	Triangle	Triangle	

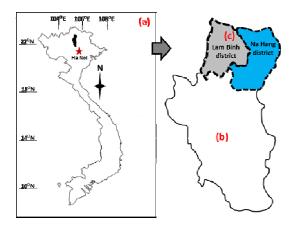


Fig. 1.(a) Map of Vietnam, (b) Tuyen Quang province, (c) Lam Binh and Na Hang districts –

253 field survey sites.



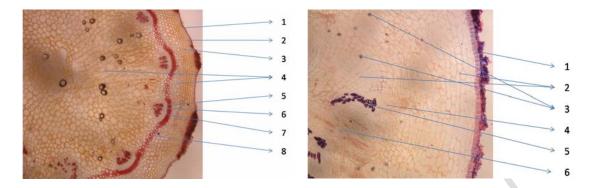
Fig. 2. Full plant of Tuyen Quang ginseng (above left), root (above right), and root transverse sections (below; light yellow in left and violet in right).





(4), and bract (5).

Fig. 3. Aerial stem (1), sub-leaves (2) Fig. 4. Inflorescence (1), bract (2), palea (3), sepal back side of leaf (3), front side of leaf (4), petal (5), stamen (6), pistil (7), stigma splits (8), sruit lengthwise (9), fruit (10).



xylem (6), phloem (7), xylem ray (8).

Fig. 5. Transverse section of aerial stem. Fig. 6. Transverse section of root. Epidermis Cutin (1), epidermis (2), collenchyma (3), (1), axialparenchym (2), clusters of calcium axialparenchym (4), sclerenchyma (5), oxalate (3), phloem (4), xylem (5), xylem rays (6).