Study on nutrient compositions of and processed products developed from Son Tra (*Docynia indica* (Wall.))

Second part

In-depth study of bioactive substances in son tra fruit and its processed product development

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I. BACKGROUND AND OBJECTIVE OF THE STUDY

Tao meo is the local name of the H'mong apple. Other names include taorung, maccam, macsamcha, and son tra. The scientific name of this species is *Docynia indica* (Wall.) Decne or *Pyrus indica* (Wall.) and belongs to the family Rosaceae. Son tra is different from other species distributed in China (*Crataegus cuneata* Sieb.et Zucc. and *Crataegus pinnatifida* Bunge - north son tra), in Europe (*Crataegus oxyacantha* L.). These other species have not been found in Vietnam [1-3]. Son tra fruits are usually harvested around September and October [3].

Son tra is found in India, Myanmar, and some southern provinces of China [3]. In Vietnam, son tra is distributed mainly in the northern, mountainous provinces such as Son La (Bac Yen, Muong La), Lai Chau (Sin Ho, Phong Tho, Tuan Giao-Pha Din), Ha Giang (Dong Van, Meo Vac, Quan Ba), Lao Cai (Sapa, Bac Ha, Muong Khuong), Yen Bai (Mu Cang Chai, Tram Tau). It grows at elevations above 1000 masl and particularly well at 1500-1700 masl [3].

Son tra has been traditionally used in supplement remedies to aid in stimulating digestion, appetite, treating bloating, and heartburn [3]. It can also be used in combination with other herbals to treat tonic spleen and digestion problem. According to the local knowledge, son tra has high nutrient values and contains substances that are essential for the human body. Despite this common knowledge, there is not yet much scientific evidence to prove the effects of son tra on human health.

Results of the first part of the study show that son tra fruits are rich in both macro- and micronutrients. Its nutrient compositions include macronutrients such as carbohydrates, proteins, fats, free sugars - monosaccharide (fructose and glucose), disaccharide (maltose and saccharose), and micronutrients - vitamin C and beta-carotene, fatty acids (palmitic acid, linoleic acid, oleic acid, linolenic acid, stearic acid, arachidic acid, and other fatty acids), amino acids (essential amino acids and nonessential amino acids), and some essential minerals (calcium, iron and phosphorus). The nutritional content of the son tra fruit is similar to that of oranges, lemons, and apples.

Phytochemical screening of son tra samples showed the presence of polyphenol (flavonoid, tannin...), saponin, organic acid, amino acid, and reducing sugar. Among those, polyphenol and organic acid groups appeared at the highest levels. Polyphenol has long been known to have antioxidant properties.

Since son tra cannot be consumed directly in a raw form, it requires processing. Manufactured, ready-for-use products could potentially increase the demand of son tra and promote its production. The development of easy-to-use products such as filtered tea bags, agglomerated instant tea, and dry jam from son tra seem to have market potential.

This part of the study focus on: (1) in-depth evaluation of some important chemical compositions of son tra such as triterpene acid, polyphenol, and organic acids; and (2) preparation of some semi-finished products from son tra (in water extract form) as pre-product for producing instant tea, son tra gel, and their standardization.

Analysis was conducted at a quality analysis laboratory at the Department of Analytical Chemistry and Standardization, of the National Institute of Medicinal Materials in Hanoi, Vietnam.

II. MATERIALS AND METHODS

2.1 Materials

Docynia indica (W.) Decne fruit samples were collected in Yen Bai, Son La ,and Dien Bien province between August and September 2013 and 2014.

Instruments

The instruments used in this study are listed in Table 1.

Table 1: List of instruments used in the study

No	Instruments	Origin
1	HPLC system	Shimadzu (Japan)
2	TLC system	Camag (Switzerland)
3	UV-1800 Spectrophotometer	Shimadzu (Japan)
4	Rotary vacuum evaporation system	Buchi (Switzerland)
5	Vacuo incubator	Binder (Germany)
6	Analytical balance	Precisa (Switzerland)
7	Ultrasonic Bath	Germany
8	Common laboratory glass	Germany
9	Scales	Germany
10	Stainess steel sieve	China
11	BladeMixer	Germany
12	Stainess steel Basin	Germany
13	Dryer	Germany
14	Granules extruding machine	China
15	Package machine	Germany

Chemicals

The chemicals used in this study are listed in Table 2.

Table 2: List of chemicals used in the study

No	Chemicals	Origin

No	Chemicals	Origin
1	Methanol	Merck (Germany)
2	Ethyl acetate	Merck (Germany)
3	Formic acid	Merck (Germany)
4	Redistilled water	National Institute of Medicinal Materials, Vietnam
5	Sulfuric acid	Merck (Germany)
6	TLC plate Kieselgel 60 F_{254}	Merck (Germany)
7	Chlorogenic acid	Sigma – Aldrich (purity, 97.0 %)
8	Gallic acid	Sigma – Aldrich (purity, 98.0 %)
9	Folin reagent	Merck (Germany)
10	Sodium hydroxide	Merck (Germany)
11	Phenolphthalein	Merck (Germany)
12	Acetonitrile	Merck (Germany)

2.2 Methods

2.2.1 Identification of the most important chemical groups in son tra fruits by TLC

The main phytochemical groups present in son tra fruits, such as triterpene acids and polyphenols were extracted by using suitable solvents such as ethanol, n-hexan, chloroform, ethyl acetate, and water. They were screened by using the thin-layer chromatography method based on the methods described in (Vietnamese Pharmacopoeia; fourth edition, Appendix 5.4) [1].

Son tra extract fraction was put in vacuum to create a concentrated fraction, which was then analyzed by TLC method, using silica gel G as the coating substance.

- *Identification of triterpene acids group in son tra fruits*

Solvent system: Ethyl acetate: methanol: formic acid: water (25:1:1:1)
Reagent: H_2SO_4 10 %/ethanol;

Detection by day light after aspray the reagent (Fig B) and UV - 365 nm (Fig C).

- *Identification of polyphenol group in son tra fruits:*

Using ethyl acetate fraction as test solution;

Solvent system: ethyl acetat: methanol: nước (10/1/1)
Reagent: Acid boric/acid oxalic (1/2) 10%/H_2O
Detection by UV - 365 nm

Prepare a solution of 1.0 g of son tra with ethanol as the reference solution. Dissolve chlorogenic acid in methanol to produce a solution containing 1mg per ml as the reference solution.

Solvent system: Ethyl acetate: methanol: formic acid: water (25:1:1:1)

Reagent: H_2SO_4 10 %/ethanol

Detection by UV - 365 nm

2.2.2 Quantitative determination of some principle chemical groups in son tra fruits

Water-soluble extractives:

The method to determine water-soluble extractives was carried out according to Vietnamese Pharmacopoeia (fourth edition, Appendix 12.10, the hot extraction method), using water as solvent. Two grams of the son tra powder was boiled with 50ml water for three hours. This was repeated two times. The solution was then rapidly filtered through filter paper and 25ml of water evaporated. The residue was dried at 105°C, weighed, and kept in desiccators. The extract was dried to a constant weight, and, finally, the % W/W of water soluble extractive value was calculated with reference to the air dried son tra fruits. Data displayed in Table 3 shows the mean of triplicate experiments.

Organic acids

Two grams of the analytical sample was soaked in 100 ml of water for four hours with occasional shaking, and was then filtered. To 25 ml of the filtrates, 50 ml of water, 2 drops of phenolphthalein, tritrate with sodium hydroxide (0.1 mol/L) was added and the content calculated. Each ml of sodium hydroxide (0.1 mol/L) is equivalent to 6.404 mg of citric acid ($C_6H_8O_7$) [2].

Total phenolic content

TPC was identified by using ultraviolet spectrophotometry and colourimetry (Vietnamese Pharmacopoeia; fourth edition, Appendix 4.1) [1]

Reference solution: 5.0 mg gallic acid, dissolved in methanol to produce a solution containing 1mg per ml.

Test solution: 2.000 g son tra sample was extracted in a mixture of methanol and water (4:1) for 30 minutes, two times. Extracts were then placed in a 50ml volumetric flask and solvent was added until the solution reached the mark. The extract was then filtered through 0.45 micron filter paper. To 0.1 ml of test solution or reference solution, 9.3 ml solution of Na_2CO_3 2% in water and 0.5 ml of Folin-Ciocalteu's reagent was added. The concomitantly blank sample was prepared, containing 0.1 ml mixture of methanol and water (4:1), 9.3 ml solution of Na_2CO_3 2% in water, 0.5 ml of Folin-Ciocalteu's reagent. The samples were placed in an incubator at 40^oC for 60 min. The absorbance was measured at λ = 760 nm [3].

The total phenolic content (%) in analytical sample was calculated with the formula:

$$X\ (\%) = \frac{A_1 \times C_2 \times 100 \times P \times 100 \times 50}{A_2 \times 1000 \times m \times (100\text{-}B) \times 100}$$

X: Total phenolic content in % calculated as gallic acid $C_7H_6O_5$

A_1: Absorbance of test solution

C_2: Concentration of gallic acid (mg/ml)

A_2: Absorbance of reference solution

m: mass of analytical sample (g)

P: Purity of gallic acid (%)

B: water content in analytical sample (%)

III. RESULTS AND DISCUSSIONS

3.1. Identification of the most important chemical groups in son tra fruits by TLC

Identification of triterpene acids

As shown in Figure 1, all three solvent fractions of son tra samples have pink colour spots corresponding with triterpene acids. This result shows that son tra sample contains triterpene acids in n-hexan, chloroform, and ethyl acetate fractions. This result also suggests a method to isolate triterpene acids from son tra fruits.

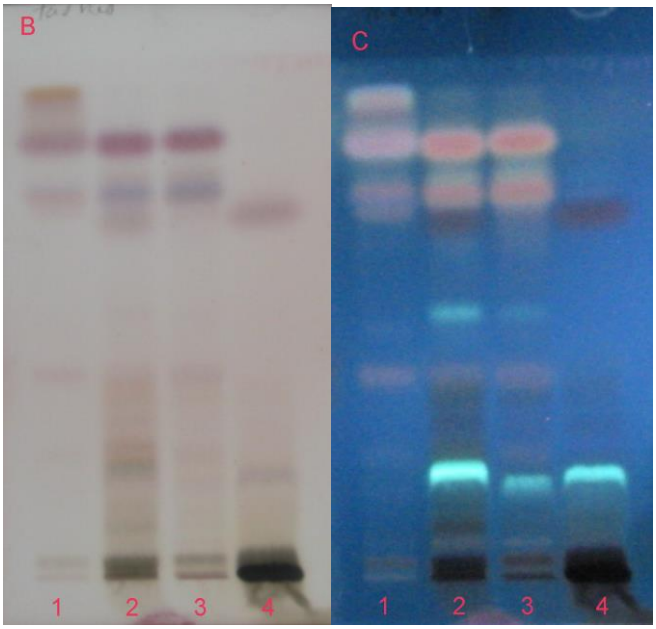

Figure 1: TLC chromatogram of the *Fructus Docyniae indicae* extract

Recently, triterpene acid (such as ursolic acid, oleanolic acid) has been shown to have various pharmacological effects such as anti-inflammation, hepatoprotective, analgesic, antimicrobial, immunomodulatory, diuretic, antispasmodic, anti-atherosclerotic... [4-5]. Ursolic acid has reportedly had an anti-obesity effect. Triterpene acids are also the main chemical group as well as the active compound in hawthorn fruits.

Identification of polyphenol group in son tra fruits

The results can be seen in the Figures 2 and 3.

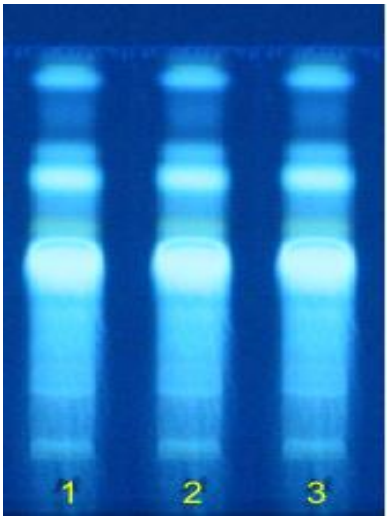

Figure 2: TLC chromatogram of *Docynia indica fruit* extract

(1, 2, 3: Ethyl acetate fraction of son tra samples in Dien Bien, Son la and Yen Bai province)

Results show that ethyl acetate fraction of son tra samples in all three provinces have the spots fluorescence (phenolic group) (Fig. 2)

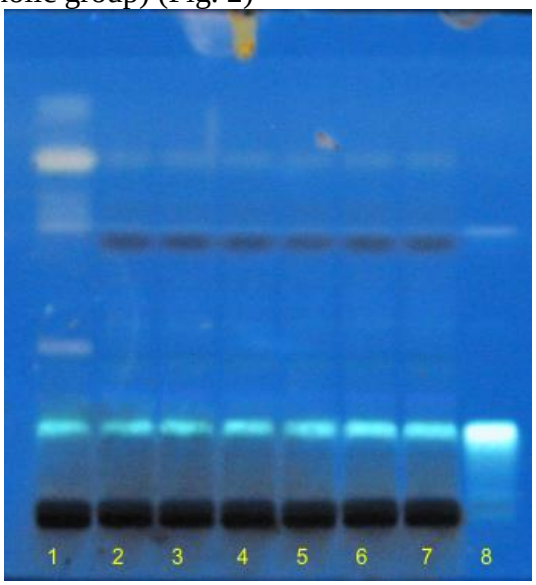

Figure 3: TLC chromatogram of Docynia *indica fruit* extract

As shown in Figure 3, all spots of the testing extracts correspond in position and colour with the spots in the chromatogram obtained from the reference sample and the marker compound (Chlorogenic acid). The spots from tested samples were separated clearly.

Chlorogenic acid is a natural compound found in hawthorn as well as in many other plants. This compound, known as an antioxidant, can also slow the release of glucose into the bloodstream after a meal.

The qualitative results of son tra extract collected in Dien Bien, Son La, and Yen Bai show that the compositions of *Docynia indica* (W.) Decne fruit extract consist of triterpene acids, polyphenol, and acid chlorogenic. However, phenolic compounds were found primarily in the ethyl acetate and ethanol fractions, triterpene acids mainly present in less polar fraction.

3.2 Quantitative determination of principle chemical groups in son tra fruits

Water-soluble extractives:

The results in Table 3 indicate that the water-soluble extractives content of son tra fruit samples range from **45.55 to 51.74%** (calculated the % W/W of water soluble extractive value with reference to the dry weight basis). Data displayed in Table 3.

Table 3: Water-soluble extractives of the *Docynia indica fruit* samples

No	Origin	Sign	Extractives (%)
1	Dien Bien	DB-1	48.18
2	Dien Bien	DB-2	51.28
3	Son La	SL-1	49.37
4	Son La	SL-2	45.55
5	Yen Bai	YB-1	46.28
6	Yen Bai	YB-2	51.74

Water soluble extractives value are the total value of those substances contained in water soluble extract, such as tannins, sugars, or plant acids. These results helps reveal the nature of chemical constituents present in the son tra fruits and also helps calculate the extraction yield of semi-finished products from son tra (in water extract).

Organic acid content

Each sample was analyzed three times. The organic acids content of six investigated samples calculated as citric acid are presented in Table 8.

Table 4: Organic acids content of the *Docynia indica* fruit samples

No	Origin	Sign	Organic acids content (%)
1	Dien Bien	DB-1	3.17
2	Dien Bien	DB-2	3.28
3	Son La	SL-1	2.85
4	Son La	SL-2	3.12

5	Yen Bai	YB-1	3.51
6	Yen Bai	YB-2	3.07

The organic acids content of dry son tra fruits varied from 2.85 to 3.51% (calculated as citric acid on a dry weight).

Organic acids of *C. pinnatifida* mainly include phenolic acids and other organic acids. Phenolic acids include benzoic acid, chlorogenic acid, and gallic acid. Other organic acids include malic acid, citric acid, quinic acid, pyruvic acid, tartaric acid, succinic acid, fumaric acid, and ascorbic acid.

Organic acids in fruits and vegetables are mostly in the form of compounds such as salt, ester, and glycoside [6]. Since acids in fruits are immediately oxidized, they do not have harmful effects on the body. As their salts are alkaline, they play an important role in human nutrition [6]. Hawthorn species have recently risen in significance in nutrition and nutraceuticals due to their beneficial effects in preventing cardiovascular diseases [7]. Quality parameters for hawthorn fruits include fruit acids that enable digestion of nutrients and stimulate blood circulation.

Total polyphenol content (Total phenolic content)

Table 5 presents total phenolic compounds content of water extracts in six son tra samples. Data displayed as mean ± SD of triplicate experiments.

Table 5: Total phenolic content of the *Docynia indica* fruit samples

No	Origin	Sign	Total phenolic content (%)
1	Dien Bien	DB-1	1.62
2	Dien Bien	DB-2	1.85
3	Son La	SL-1	1.37
4	Son La	SL-2	1.41
5	Yen Bai	YB-1	2.31
6	Yen Bai	YB-2	2.17

The analytical results of total phenolic content showed that TPC concentrations varied from 1.37 % to 2.31 % (calculated as gallic acid on a dry weight basis samples). This value is lower than the content in *C. monogyna* fruits (2.83% - calculated as gallic acid on a dry weight basis samples).

3.3. Product development from son tra fruit

3.3.1 Preparing semi-finished products from son tra (water extract)

Extraction procedure

At the first stage**,** dried *Docynia indica* fruits were cut into small pieces and extracted twice with boiling water. The first time with 30 liters and the second with 20 liters water for eight hours. The extract was the combined and removed from a solvent to obtain liquid extract (10 liter)**,** which was diluted into 20 liters 96% ethanol, precipitated, and filtered to obtain a solution. The mixture was then further removed from solvent at 60 $^{\circ}$C under low pressure. Finally, the residue is dried at 60 $^{\circ}$C in vacumn to obtain the semi-finished product: son tra gel (Figure 4).

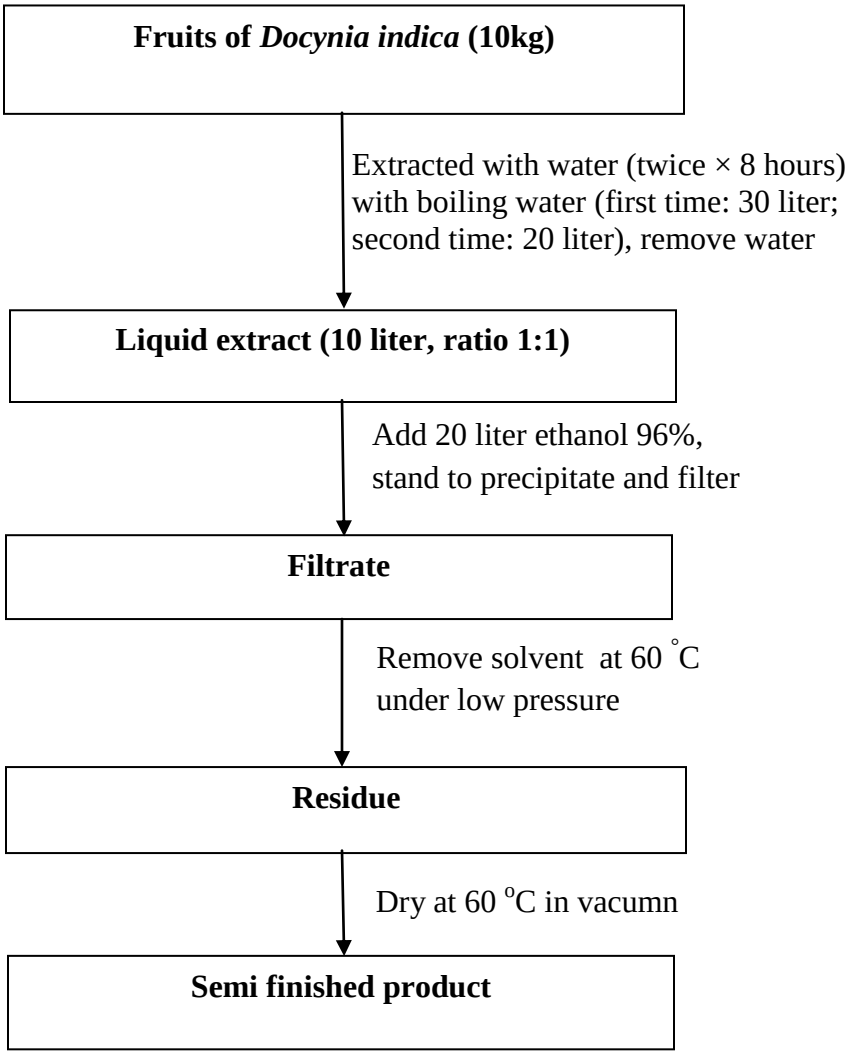

Figure 4. Extraction procedure of *Docynia indica* extract

Extraction Yield

Table 6: Yield of son tra extraction

No	Weight of son tra materials (kg)	Fruit of *Docynia indica* extract		
		Weight of extract (kg)	Moisture (%)	Extraction Yield (%)
1	10.0	1.08	15.27	9.23
2	10.0	1.26	16.38	11.10
3	10.0	1.36	18.32	12.46
4	10.0	0.93	15.74	8.26
5	10.0	1.41	16.25	10.82
Average	10.0	1.25	16.39	10.37

The son tra extract is a soft gel, dark brown in colour, aromatic, and has an acrid and sour taste**.** The above table shown that from 10kg of son tra fruits, 1.25 kg son tra gels (with moisture-16.39%) can be produced so that the extraction yield was about 10.37%.

3.3.2 Product development from son tra fruit

Son tra agglomerated instant tea

Instant (or soluble) medicinal plant tea has been widely used for decades because of its convenience. The product is easy to use, has a simple production technology, and is low in price. Son tra instant tea is produced from son tra extract/gel by wet granulation method. Wet granulation process simply involves wet massing of the powder blend with a granulating liquid, wet sizing and drying. The steps follow (Figure 5):

- Mix son tra extract /gel with excipients,
- Mix binder solution with powder mixture to form wet mass
- Coarse screening of wet mass using a suitable sieve
- Drying of moist granules
- Screening of dry granules through a suitable sieve
- Mix screened granules with lubricant

Table 7: Content of a son tra tea bag

Item	Material name	Mass in mg or bag
1	Son tra Extract	1200
2	Folium *Steviae rebaudianae* Extract	10
3	Excipients	3790

Characteristic of son tra instant tea

- Dosage form: granule
- Taste: sweet
- Specifications: in-house

Product standardization

Standardization is used to assess the quality of the son tra fruit, son tra extract, and son tra instant tea. Three monographs for this purpose were completed, including the characteristics such as description, water content, total ash, identification, water soluble extractive and assay of total phenolic, chlorogenic acid, and organic acid content.

VI. Summary and conclusions

Evaluation of the important chemical compositions of son tra

The aim of this study was to characterize the important nutrition composition as well as other phytochemical components contributing to the nutritional profile and health benefit of *Docynia indica* (Wall.) Decne fruits. Phytochemical screening of son tra fruits showed the presence of substances such as polyphenol, tannin, saponin, organic acid, amino acid, and reducing sugar, which are essential to human nutrition. TLC identification of son tra fruits confirmed the presence of triterpene acids, polyphenol, and chlorogenic acid, which are active compounds in the famous Hawthorn (North son tra). These results also provide evidence supporting theuse of Vietnamese son tra as an alternative to North son tra, which is imported from China.

In developing ready-to-use son tra products, we focused on the traditional of herbal medicine to produce nutritional supplements that serve as an antioxidant as well as supporting treatments for obesity and cardiovascular disease. Because the health benefits connected to **polyphenolic compounds** and **triterpene acids** are well known [8], these active groups were analyzed qualitatively and quantitatively. The results of the analysis are used in the preparation of *Docynia* fruits, the products' monograph, and the development of pharmacopoeial standards.

Product development from son tra fruit

As discussed above, son tra fruit cannot be consumed directly after purchase. The fruit requires specific processing, which makes son tra fruits inconvenient for many consumers. The production of ready-to–use son tra products would increase the consumption of son tra and thus promote son tra production. Moreover, a growing number of son tra plantations have increased the production and supply of the fruit. The development of processed son tra products adds value to the fruit production and prepares the market to receive the increased supply.

In this study, the method and standardization of preparing semi-finished son tra products (water extract/gel) as a pre-product for instant tea and solid extracts was tested. The development of high-quality, instant products can contribute to the popularization of the herbal tea. The extraction and purification methods to produce son tra instant tea have proven to be simple, quick, and efficient. The identified process creates a foundation for further study and up-scaling the extraction method.

In summary, the most significant essential chemical substances identified in son tra fruits include triterpene acids, polyphenols, and organic acids. The tested son tra products, instant tea and son tra gel, proved to have low cost, low investment, and require simple technology. Prior to mass production, a study on consumer reception of the developed products is recommended.

REFRENCES

1. Cây Thuốc và Động Vật làm thuốc ở Việt Nam, nxb KH&KT Hà Nội, 2006, pp. 785-787.
2. Võ Văn Chi, Từ Điển Cây Thuốc Việt Nam, nxb Y Học, 1997, pp. 1097-1098.
3. Đỗ Tất Lợi, Những Cây Thuốc và Vị thuốc Việt Nam, nxb Y học, 2004, pp. 355-357.
4. Scalbert A, Manach C, Morand C, Remesy C. Dietary polyphenols and the prevention of diseases. Crit Rev Food Sci Nutr. 2005;45:287–306.; Pandey K B nd Rizvic S I, Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev. 2009 Nov-Dec; 2(5): 270–278.
5. Volz RK, McGhie TK: Genetic Variability in Apple Fruit Polyphenol Composition in Malus × domestica and Malus sieversii Germplasm Grown in New Zealand. Journal of Agricultural and Food Chemistry 2011, 59:11509-11521.
6. Landete, J. M. Updated knowledge about polyphenols: functions, bioavailability, metabolism, and health. Crit. Rev. Food Sci. Nutr. 2012, 52, 936−948.
7. Mahato SB. *et al*. (1988) Triterpenoid saponins, *Phytochemischy*, 27, 3037-3067.
8. Safayhi H, Sailer ER. (1997), Anti-inflamatory actions of pentacyclic triterpenes, *Planta Med*, 63, 487-493.
9. Babalola IT, Shode FO. (2013), Ubiquitous ursolic acid: A potential pentacyclic triterpene natural product, *Journal of Pharmacognosy and Phytochemistry*, 2(2), 214-222.
10. Wang Y, He Y. (2014), Ursolic acid, a promising dietary bioactive compound of anti-obesity, *The FASEB Journal*, 28(1), 1045.40.
11. Kim J. *et al*. (2009), Anti-lipase and lipolytic activities of ursolic acid isolated from the roots of *Actinidia arguta*, *Arch Pharm Res.*, 32(7), 983-987.
12. Mark Kern, CRC desk reference on sports nutrition. CRC Press. 2005, pp. 117–120.
13. Mark Kern, CRC desk reference on sports nutrition. CRC Press. 2005, pp. 121–122.
14. Whitney, Elanor and Sharon Rolfes, Understanding Nutrition, Thomson-Wadsworth Press, 10th Ed., 2005, pp. 6. 2. Frances Sizer, Ellie Whitney, Nutrition: Concepts and Controversies. Thomson Wadsworth Press, 11 Ed., 2007, pp. 6-28. 11. http://www.unicef.org/nutrition/training/4.1/10.html.
15. Bộ môn Dược liệu, Thực tập dược liệu, Trường Đại học Dược Hà Nội, 1999.
16. Viện dược liệu, Nghiên cứu thuốc từ thảo dược, giáo trình sau đại học, nxb KH&KT, 2006.
17. Harborne J. B. Phytochemical Methods: A guide to modern techniques of plant analysis. Chapman & Hall, New York, 1973.
18. Claudia A., Graciela E. F., Rosana F. Total polyphenol content and antioxidant capacity of commercially available Tea (*Camellia sinensis*) in Argentina J. Agric. Food Chem. 2008, 56, 9225–9229
19. Ministry of Health (2009), *Vietnamese Pharmacopoeia*, fourth edition, Medical publishing House.
20. Chinese pharmacopoeia Commission (2010), *Chinese pharmacopoeia*, China Medical Science and Technology Press, Vol 1, 135.
21. Mraihi F. et al. (2013), Phenolic contents and antioxidant potential of *Crataegus* fruits grown in Tunisia as determined by DPPH, FRAP, and β-Carotene/Linoleic acid Assay, *Journal of Chemistry*, 2013, 1-6.
22. The British Pharmacopoeia Commission, British Pharmacopoeia, 2009.

23. Gambhava NS. et al. (2013), Estimation of ursolic acid and oleanolic acid from leaves of *Plumeria obtusa* by HPTLC method after iodine derivatization, *Der Pharma Chemica*, 5(3), 44-50.
24.T. Bahorun, F. Trotin, J. Pommery, J. Vasseur, and M. Pinkas, "Antioxidant activities of Crataegus monogyna extracts," Planta Medica, vol. 60, no. 4, pp. 323–328, 1994. View at Publisher · View at Google Scholar · View at Scopus
25. T. Bahorun, B. Gressier, F. Trotin et al., "Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical preparations," Arzneimittel-Forschung, vol. 46, no. 11, pp. 1086–1089, 1996. View at Scopus