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Secondary metabolites in *Pseuderanthemum palatiferum*

Bachelor thesis

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Thesis supervisor:

Ing. Olga Leuner, Ph.D.

Elaborated by:

Veronika Janoušková

CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

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BACHELOR THESIS ASSIGNMENT

Veronika Janoušková

Agriculture in Tropics and Subtropics

Thesis title

Secondary metabolites in *Pseuderanthemum palatiferum*

Objectives of thesis

People have been using natural herbal treatments for thousands of years to mitigation or disappearance the symptoms of the disease. In some countries this tradition has remained till today, for example in Vietnam, where *Pseuderanthemum palatiferum* come from. This plant has been used to treat many health disorders, but the scientific evidence to advocate the uses is missing. Therefore the aim of this thesis is to review the secondary metabolites present in this plant with possible biological activities.

Methodology

Literature review containing information from scientific web databases (Web of Knowledge, Google Scholar, etc.), scientific publication, journals and books.

The proposed extent of the thesis

30 pages

Keywords

lifestyle diseases, Vietnam, traditional medicine, medicinal plants, plant extracts

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Recommended information sources

Dieu H, Loc C, Yamasaki S, Hirata Y. 2005. The ethnobotanical and botanical study on Pseuderanthemum palatiferum as a new medicinal plant in the Mekong Delta of Vietnam. JARQ-JAPAN AGRICULTURAL RESEARCH QUARTERLY 39:191–196.

Chayarop K, Peungvicha P, Wongkrajang Y, Chuakul W, Amnuoypol S, Temsiririrkkul R. 2011. Pharmaconostic and phytochemical investigations of Pseuderanthemum palatiterum (Nees) Radlk. ex Lindau leaves. Pharmacognosy Journal 3:18–26.

Nguyen QV, Eun J. 2011. Antioxidant activity of solvent extracts from vietnamese medicinal plants. Journal of Medicinal Plants Research 5:2798–2811.

Petsangkrit N, Kittipongpatana N. 2016. Establishment of pseuderanthemum palatiferum (nees) radlk callus culture and screening of secondary metabolite production. International Journal of Pharmacy and Pharmaceutical Sciences 8:275–280.

Pulbutr P, Nualkaew S, Rattanakiat S, Cushnie B, Jaruchotikamol A. 2016. Inhibitory actions of Pseuderanthemum palatiferum (Nees) Radlk. leaf ethanolic extract and its phytochemicals against carbohydrate-digesting enzymes. Asian Pacific Journal of Tropical Biomedicine 6:93–99.

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The Bachelor Thesis Supervisor

Ing. Olga Leuner, Ph.D.

Supervising department

Department of Crop Sciences and Agroforestry

Electronic approval: 29. 3. 2018

doc. Ing. Bohdan Lojka, Ph.D.

Head of department

Electronic approval: 4. 4. 2018

doc. Ing. Jan Banout, Ph.D.

Dean

Prague on 19. 04. 2018

Declaration

I, Veronika Janoušková, declare that I have elaborated my thesis "Secondary metabolites in *Pseuderanthemum palatiferum*" independently and quoted only quotations listed and references.

In Prague, 20th April 2018 …….

Veronika Janoušková

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I am very thankful to my supervisor Ing. Olga Leuner, Ph.D. (Department of Crop Sciences and Agroforestry, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague) for her valuable instructions and suggestions and mainly for her endless patience.

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And the last person I want to thank is my boyfriend, who protected me against the wild growing weed.

Abstract

Pseuderanthemum palatiferum (Nees) Radlk. is evergreen shrub known in the southeast of Asia as Hoan-Ngoc, Wan ling or Payawanorn. It has a long tradition especially in Vietnam and Thailand as a medicinal plant. In this literature review are described current knowledge of taxonomy, planting, chemical compounds and traditional uses of this species. In Hoan-Ngoc are contained several chemical compounds from secondary metabolites as terpenoids (phytosterols), phenylpropanoids (phenolic acids, flavonoids), amino acids and several minerals. Consequently, contained chemical substances were detected and described from different parts of the plant, especially from leaves or stems, with separated, spectroscopic and radioactive methods. Chemicals proved in the phytochemical profile of *P. palatiferum* are reported an anti-inflammatory and antioxidant potential and good influence of cardiovascular system, skin diseases, growth and recovery of human tissues and even on the treatment of animal diseases connected with digestive system (diarrhea or cholera). Due to the pharmacological activity of chemical substances reported in *P. palatiferum* is possible to use fresh parts of this species or dietary supplements containing dry or powder parts of Hoan-Ngoc to treatment or prevention against lifestyle diseases.

Key words: ethnobotany, Vietnam, traditional medicine, medicinal plants, secondary metabolites

Abstrakt

Pseuderanthemum palatiferum (Nees) Radlk. je stálezelený keř známý v jihovýchodní Asii jako Hoan-Ngoc, Wan ling nebo Payawanorn. Obzvláště ve Vietnamu a v Thajsku je spojený s dlouholetou tradicí jako léčivá rostlina používaná v tradičním léčitelství. Tato literární rešerše popisuje dosud známé údaje o *P. palatiferum* - taxonomii, možnosti pěstování, chemické složení a užití v tradiční medicíně. Co se týče chemického složení, jsou zde obsaženy zástupci z řad sekundárních metabolitů, jako jsou terpenoidy (fytosteroly), fenylpropanoidy (flavonoidy, fenolové kyseliny), aminokyseliny a řada minerálů. Látky obsažené v rostlině, hlavně tedy v listech a stonku, byly rozpoznány a popsány za pomoci separačních, spektroskopických a radioaktivních metod. Chemická analýza poukázala na antioxidační a protizánětlivý potenciál testovaných látek a jejich následný příznivý vliv na kardiovaskulární systém, léčbu kožních onemocnění, růst a obnovu lidských tkání a dokonce na léčbu onemocnění zvířat spojených se zažívacím ústrojím (průjmové onemocnění nebo cholera). V důsledku farmakologické aktivity chemických látek obsažených v *P. palatiferum* je možné její užití k prevenci nebo následné léčbě civilizačních onemocnění.

Klíčová slova: etnobotanika, Vietnam, tradiční medicína, léčivé rostliny, sekundární metabolity rostlin

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List of abbreviations

c. = corolla

PP = *Pseuderanthemum palatiferum*

kg/1000 m^2 = kilogram per 1000 square meters

cm = centimeters

mg/ 100g = milligram per 100 gram

EtOAc = ethyl acetate

EtOH = ethanol

$CHCl_3$ = chloroform

MeOH = methanol

H_2O = water

MPLC = medium pressure liquid chromatography

UV = ultraviolet

VIS = visible spectrometry

nm = nanometers

µm = micrometers

MO = molecular orbital

NO = nuclear orbital

IR = infrared

mm = millimeters

MS = mass spectrometry

m/s = mass to charge ratio

List of abbreviations (continues)

^{1}H NMR = hydrogen nuclear magnetic resonance

^{13}C NMR = carbon nuclear magnetic resonance

1. Preface

Using food or dietetic supplements in traditional healing is used in many cultures around the world. Probably the most famous eastern state with traditional medicine is China. Many other nations have also been inspired in this country, such as Vietnam, known for its cultivation and subsequent use of medicinal herbs. Not only special plants but also "ordinary foods" such as vegetables (garlic, spinach), fruit (mango) or spices (turmeric, ginger) are used by people in eastern states to positively influenced their health. These plants are most commonly used to prevent lifestyle diseases (listed in Table 1) or to treat illnesses. However, natural medicine is not limited to humans but can also be used in farm animals or domestic animals (Opletal 2016).

Table 1 Civilization diseases (21.století 2010)

List of the most common lifestyle diseases
Atherosclerosis
Heart-attack
Stroke
Hypertension
Obesity
Diabetes mellitus
Cancer
Rheumatic diseases
Premature births, abortions
Defects of the neonatal nervous system
Alzheimer's disease
Chronic fatigue syndrome
Burnout
Depression

The reason why plants have medicinal effects is the production and accumulation the phytochemical substances with biological activity, antioxidant and anti-inflammatory effects (Opletal 2016).

Antioxidants potential have many dietetic supplements protecting the human body against the impact of free radicals. During the process of acquiring an energy are created by human's cells substances called the free radicals. These substances contain highly unstable oxygen atoms. These unstable oxygen atoms react with other atoms in

the body and consequently release the energy which can damage the human's cells and tissue. Due to it is important to use the antioxidants as the dietary supplement because with the passing of time are still more and more damaged the important tissues and organs (veins, heart, cartilages, even brain) (Mindell 1997). Anti-inflammatory products reduce swelling, heat, redness, and pain due to inflammation. Thanks to entering into the central nervous system are block the pain straight in the brain (Dr. Axe 2018).

These plant substances are really needed in today's world because following the present situation of lifestyle diseases is alarming, especially in developed countries. Therefore, it is important to list and remind this traditional knowledge for future, before it becomes lost.

2. Objectives

The main objective of this Bachelor's thesis was to summarize the phytochemical profile (secondary metabolites, minerals, and other chemical compounds) of a plant *Pseuderanthemum palatiferum* from family Acanthaceae that is commonly used in Southeast Asia against lifestyle diseases in fresh form (chewing fresh leaves) or as dietary supplements (herbal tea bags, capsules).

3. Methodology

This study was written in a form of literature review. The most important information was collected from scientific publications. This publication was reviewed from scientific web databases as Web of Knowledge, Scopus, ResearchGate, Elsevier or Google Scholar. Other sources of information were scientific journals, studies, and books. Searched publications were contained mainly topics about *Pseuderanthemum palatiferum* studies or taxonomy and secondary metabolites and their influence on human or animal health.

This Bachelor's thesis was written according to a Methodical manual for the Writing of Bachelor's Theses 2018 and also Citation Rules of the Faculty of Tropical AgriSciences, CULS Prague for FTA 2018, published by Tropical Faculty of AgriSciences 2018.

4. Literature review

a. Botany classification

- Kingdom • Plantae
- Subkingdom • Tracheobionta
- Superdivision • Spermatophyta
- Division • Magnoliophyta
- Class • Magnoliopsida
- Subclass • Asteridae
- Order • Scrophulariales
- Family • Acanthaceae
- Genus • *Pseuderanthemum*
- Species • *Pseuderanthemum palatiferum*

(US Department of Agriculture: NRCS 2017)

b. Geography

Pseuderanthemum palatiferum (also known as Hoan-Ngoc) was discovered in the forest named Cuc Phuong on the north of Vietnam. This is probably the area of natural occurrence of this plant used for medicinal treatment or prevention of disease (Rupam et al. 2012). In present can be this medicinal plant found all over the country, even in the southern region (for example in Mekong Delta region – a place for collecting leaves of *Pseuderanthemum palatiferum* for research) (Padee et al. 2010).

Cuc Phuong forest is the part of national park located in Ninh Binh Province. It was the first founded national park in Vietnam with very important and unique biodiversity (iGrandtour Corporation 2012).

About fauna – there can be beholden many species across the animal realm – from Protozoa, Vermes, Mollusca, Arthropoda, Chordata, Insecta to Mammals. Many of them are rare and not studied, yet. But life of these precious species is threatened by poaching and illegal logging. The number of killed or poaching animals is raising. But this is not the only problem ruining animal's life. There are others dangerous situations like water-loss, floods or falling rocks.

Flora contains over 2000 species of plants (TUYET 2010). The highest of them are represented by trees with height about 25-50 meters. Then there are mangroves and tropical grasses (these two groups occupy the biggest part of plants), lianas, aquilarias (*Aquilaria agallocha* - evergreen trees produced agarwood) and on the boughs are trees occurred by orchid, bromeliads, and ferns (MAHALO.cz 2017). Unfortunately, lower plants are less discovered then other. And into this group belongs *Pseuderanthemum palatiferum* (TUYET 2010).

Figure 1 Location of Cuc Phuong National Park (Straub 2006)

c. Description

I. Acanthaceae family

Groups of pseuderanthemum's genera belong to the family Acanthaceae (US Department of Agriculture: NRCS 2017). The family contains two types of plants habits – shrubs or herbs (Watson & Dallwitz 1992). The most common are perennial plants (genus *Pseuderanthemum*), rarely there are annual herbs (genus *Dicliptera*) (Grulich 2016). The herbs have two form of leaves – with basal aggregation or without conspicuous aggregation (Watson & Dallwitz 1992).

Leaves are well-developed, simple, opposite, sometimes whorl leaves, with dorsiventral or bifacial lamina (Grulich 2016). The typical leaf is thin and flat, with or without gland-dotted or epidermal salt glands, main veins embedded, minor veins with or without phloem transfer cells. Stipulates are not formed (Watson & Dallwitz 1992).

Figure 2 Opposite (a) and whorl (c) leaves (Krejčí 2008)

Flowers are solitary or made from *inflorescentia – cyma, verticillium,* and *racemus* (blooms create seemingly racemes). Under the blooms are big colorful *bracteas* in many pairs (usually 4-5) and *bracteolas*. Sometimes *bracteas* and *bracteolas* coalesce.

Blooms are tetracyclic, 4 or 5 merous, involving the perianth and androecium and quite symmetrically. *Calyx* is 5 merous, sometimes 4 merous and rarely 3 merous. *Sepals* coalesce and this type of *calyx* is called *calyx synsepalous* – differentiate *tubus calycis* and *dens calycis. Corolla* has coalesced *petalum*, too. And due to is corolla divided on *tubus corollae* and *limbus corollae* and the whole formation is called *corolla sympetalous*. In family Acanthaceae can be defined these types of *corolla – c. infundibuliformis, c. hypocrateridormis* and *c. campanulata. Stamen´*s four or five filaments accrete to *corolla* and anthers make one or two *thecae* (Grulich 2016).

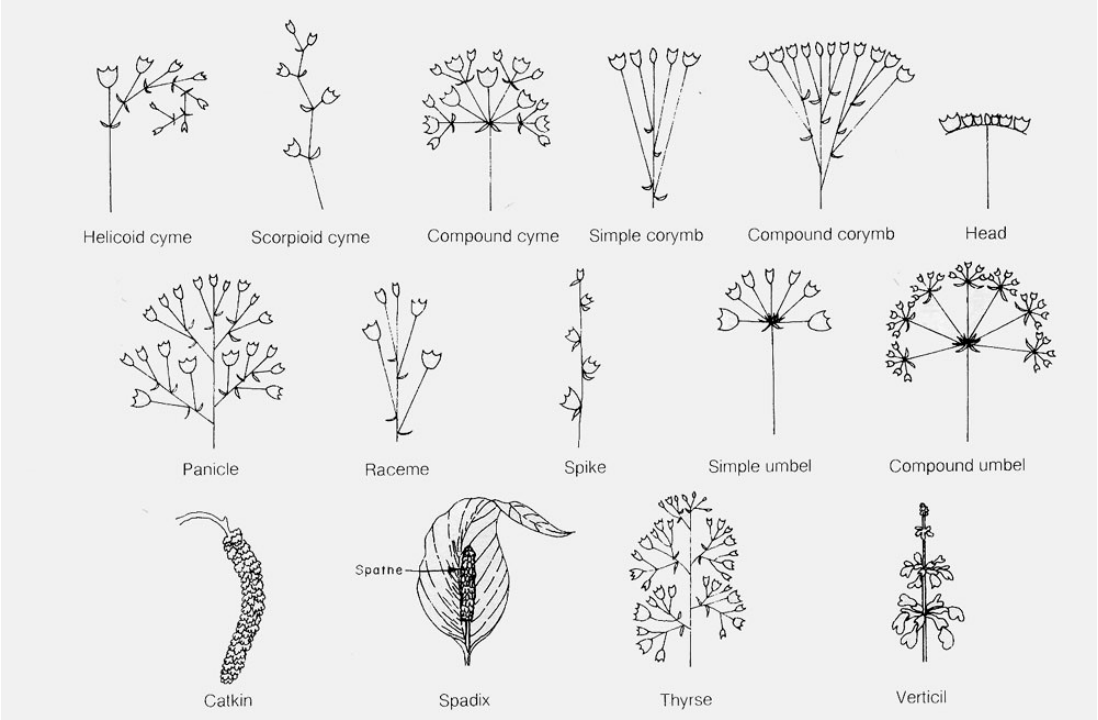

Figure 3 Cyme, raceme and verticil inflorescence (Jones & Luchsinger 1986)

Fruit of Acanthaceae is a loculicidal capsule. This type of capsule is dehiscent and non-fleshy. The capsule is made from the conversion of *carpellums*. It is a typical fruit of Angiosperms. A number of seeds in each capsule are variable – in one species contain fruit a lot of seeds but in second species we can find only two seeds (Grulich 2016).

An embryo in a seed is big and well differentiated, but the seed has not got endosperm. In this family exist polyembryony and it is very common (Watson & Dallwitz 1992).

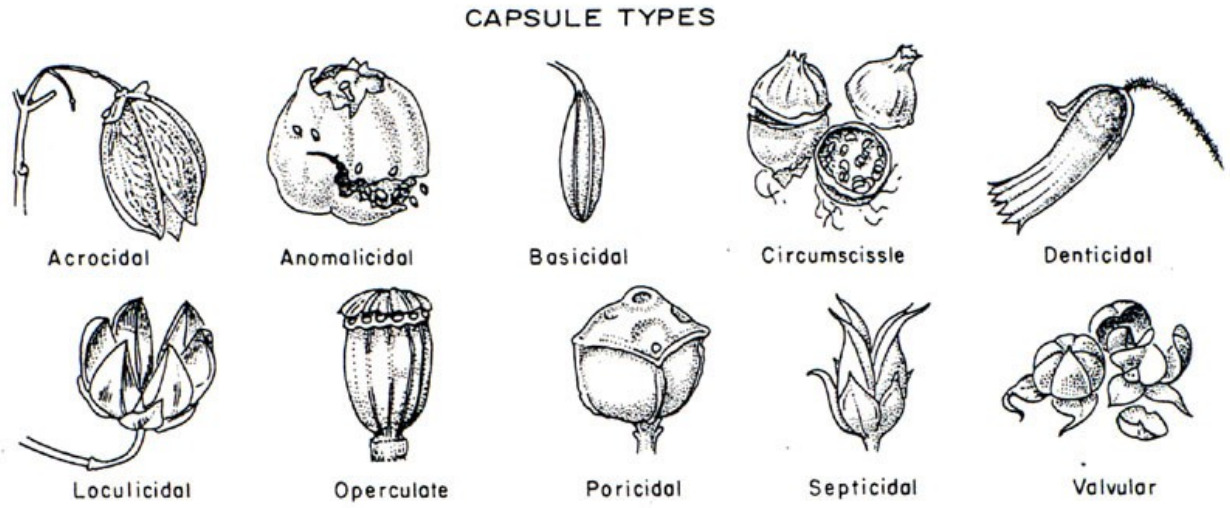

Figure 4 Capsule types (The University of North Carolina at Chapel Hill 2018)

Phytochemistry running in C3 or C4 way. C4 is typical for Afrasian species, for example, genus *Blepharis* (Watson & Dallwitz 1992).

The most common places of occurrence of Acanthaceae are dry open habitats. In many species was discovered a relation between plants and soil with heavy metals – plants indicate the presence of chemical elements (Grulich 2016).

II. Pseuderanthemum genus

Pseuderanthemum plants grow up in undergrowth in tropical forests or in places with favorable condition (for example in Mekong Delta region in Vietnam listed in Table 2) as an evergreen shrub, which is bloomed for all over the year. The height of shrub takes 1-3 m (Grulich 2016). Some of them grow as an ornamental plant in the gardens or in a tropical greenhouse, for example, *Pseuderanthemum reticulatum* or *Pseuderanthemum atropurpureum*. In gardens or greenhouses are shrubs kept of height around 1 m (Kunte & Zelený 2009).

Figure 5 *Eranthemum palatiferum* (Fitch 1872)

Branches are straight with opposite leaves (length 5-20cm, width 4-10 cm). The lamina is elliptical to oval, the top of the leaf is spiked. Blooms take about 2.5 cm, color is white to purple and they are forms in *racemus inflorescentia*. *Calyx* is green and measures 8 mm. The fruit of *Pseuderanthemum* is the capsule (Svobodová 2014).

Table 2 Growth of *Pseuderanthemum palatiferum* in Mekong Delta, Vietnam (Dieu et al. 2005)

Age in days	Number of leaves	A height of cauline (cm)	A diameter of canopy (cm)	Green yield (kg/1000 m^2)
0	3-5	8-12	-	-
14	5-8	15-20	-	-
44	100-200	40-50	30-40	-
134	700-1000	80-120	70-100	500-700

Figure 6 Leaves of *Pseuderanthemum palatiferum* (Nguyevan 2016)

Figure 7 Blooms of *Pseuderanthemum palatiferum* in raceme verticillium (The Vitaminbox 2018)

d. Planting

Pseuderanthemum genus grows in dry warm habitats with high humidity. Representatives from the species are very sensitive, ergo sufficient humidity is a necessary part of plants lives (Kunte & Zelený 2009). Some plants need light all day (especially ornamentals) and others need penumbra (for example *Pseuderanthemum palatiferum* growing in the lowest zone of the tropical forest) (Svobodová 2014).

About reproduction – *Pseuderanthemum* plants are hermaphrodites. It means flowers have both sex – androecium and gynoecium (Krejčí 2008). In sexual reproduction are needed both sex, because it became the fusion of gametes (from a male and female plant) and consequently is created a new embryo. But easier and faster for plants is an asexual reproduction, however, both choices need the right condition for successful grow an individual plant.

Asexual type of reproduction is typical with no sex cells – gametes. Plant use their rhizomes, stolons, runners, tubers, corms, bulbs and horsetails for propagation themselves. It means a new individual is a clone, and it is created from somatic cells, consequently differentiated in organs, meristems, rhizomes and then regenerated in a functional plant (Lambers 2017). In a case of *Pseuderanthemum palatiferum* is the most common cultivation for research, from the methods of asexual reproduction, termed micropropagation.

As micropropagation is called several operations using selected genotype in *in vitro* techniques. The process has five stages (from 0 to 4) and each of them is the most important for successful ending and regeneration. Equally important is the right choice for starting material. Criteria for selection are different for fruit and ornamental type and for vegetable and other types of plant. For ornamentals and fruit are important phenotype whereas for vegetable and others are watched sign of phenotype either or production of seed or biomass. About quantity of materials – can be used only one cell, but there is a higher rate of failure so it is better to use more cells. Each cell used for micropropagation need to carry the genetic information (Debergh & Read 1991).

e. Uses

Pseuderanthemum palatiferum as a medicinal plant originating from Vietnam with anti-inflammatory and antioxidant potential can be used for a treatment or prevention of many diseases – for example, diarrhea, hypertension, cancer, nerve disease, allergy, skin diseases, and so on (Dieu et al. 2006).

Due to good results of using products or extract of leaves or just fresh leaves was increasing popularity, especially in others states in Asia. Mainly in Thailand, where this plant is called "Wan ling" or "Payawanorn". But using *Pseuderanthemum palatiferum* is not limited only to people. In many villages has been this plant used for prevention and treating of animal diseases (Dieu et al. 2006).

About Hoan-Ngoc was written many studies as The ethnobotanical and botanical study on Pseuderanthemum palatiferum as a new medicinal plant in the Mekong Delta of Vietnam by Dieu et al. 2005, Anti-inflammatory activity of ethanol extract from the leaves of Pseuderanthemum palatiferum (Nees) Radlk. by Khumpook et al. 2013 or A review on medicinal plants having antioxidant potential by Rupam et al. 2012, which revealed a mixture of chemicals and different possibilities of uses (Dieu et al. 2005).

I. The chemical composition of *Pseuderanthemum palatiferum*

Plants produce primary and secondary metabolites. Primary metabolites are necessary for survival, normal growth, development and subsequent reproduction of the plant. At the first place, they ensure normal physiological function and they are produced easily, in large quantities and as molecules with low molecular weight. It is a group made of carbohydrates, lipids, proteins and nuclear acids. These molecules have participated in metabolic pathways, the Krebs cycle and the Calvin cycle (Rachna 2017).

Secondary metabolites are a metabolic waste or end product of metabolism. They are bigger molecules and not so needed, unlike primary metabolites. They can be useful as protection from animals (herbivores) and pathogens, in case of competition or symbioses and finally for reproduction – due to color, taste, and odor can look more attractive for pollinators and seed-dispersal animals. Group of secondary metabolites is created from alkaloids, terpenoids, flavonoids, glycosides, natural phenols, phenazines, polyketides or nonribosomal and ribosomal peptides (Ördög & Molnár 2011).

II. Secondary metabolites contained in *Pseuderanthemum palatiferum*

In *Pseuderanthemum palatiferum* are secondary metabolites (Table 3) deputized by 2-hydroxybenzoic acid, n-pentacosan-1-ol, stigmasterol, stigmasterol-3-O-β-glucoside, kaempferol 3- methyl ether 7-O-β-glucoside, β-sitosterol, β- sitosterol-3-0-β-glucoside, apigenin 7-0-β-glucoside and 1-triacontanol (Phan, M. G., Ha, V. B., and Phan 2003).

Secondary metabolites contained in *Pseuderanthemum palatiferum* predominantly belong to terpenoids and phenylpropanoids. From terpenoids are representatives phytosterols. Phytosterols known also as plant sterols are molecules similar to cholesterol structure but their metabolism is different. It can be found in cell membranes of plants, similar to cholesterol located in human cells. But phytosterol can lower the cholesterol levels and due to can be lower risk for heart attack, cardiovascular diseases, even cancer. Phytosterols are contained in nuts, seeds, fruits, vegetables, legumes, vegetable oils and cereal grains and in *Pseuderanthemum palatiferum* are represented by β-sitosterol and stigmasterol. (Leech 2015).

From phenylpropanoids are representatives from phenolic acids and flavonoids. Phenolic acids are type of phytochemical from polyphenols contained in fruits (mango, berries, apples, citrus fruits), vegetables (onion) and grains (wheat, rice, corn, oats) and acted as the antioxidant (protect human cells against the damage of free radicals. In nature is phenolic acids divided into two types of chemicals – benzoic acid derivatives and cinnamic acid derivatives (Lehman 2016). In *Pseuderanthemum palatiferum* are represented benzoic acid derivatives by salicylic acid (Phan et al. 2003). To polyphenols belong not only phenolic acids but even flavonoids contained in *P. palatiferum* too. Flavonoids are also known as categories including flavonoids, flavones, flavonones, flavan-3-ols, and anthocyanidins. It is substances used mainly for antioxidant and anti-inflammatory benefits to support cardiovascular and nervous system. Flavones are mainly contained in vegetables (onions, parsley, bell peppers, tomatoes and so on), fruits (apples, oranges, blueberries, bananas, lemons, strawberries, etc.), nuts (almonds), beans and legumes (garbanzo beans, quinoa) (Mateljan 2018).

Table 3 Secondary metabolites contained in leaves of *Pseuderanthemum palatiferum* (Phan et al. 2003)

Secondary metabolites
2-hydroxybenzoic acid
β-sitosterol
n-pentacosan-1-ol
stigmasterol
β-sitosterol 3-O-β-glucoside
stigmasterol 3-O-β-glucoside
kaempferol 3-methyl ether 7-O-β-glucoside
apigenin 7-O-β-glucoside

2-hydroxybenzoic acid ($C_7H_6O_3$) also known as salicylic acid belongs to phenolic acid, especially to lipophilic monohydroxybenzoic acid. It is transformed from salicin (β-glucoside and alcohol) – chemicals with antioxidant, anti-inflammatory and antipyretic potential as salicylic acid too. Close to this phenolic acid are aspirin with similar structure but different metabolism and method of use. Salicylic acid is commonly used for keratoplastic effect – in low concentration encourages skin epithelium to grow, in higher concentration causes peeling of the skin, for example, peel off warts. In skincare products is used for treating skin diseases including acne, seborrheic dermatitis, dandruff, dermatophytosis, hyperpigmentation, warts and so on. It can be found in other plants as *Salix alba* or *Spiraea ulmaria* (DRUGBANK 2005).

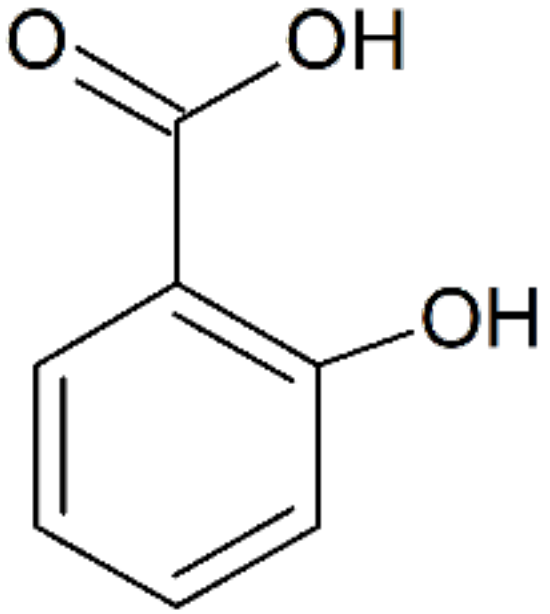

Figure 8 Salicylic acid

Another secondary metabolite contained in *Pseuderanthemum palatiferum* is β-sitosterol ($C_{29}H_{50}O$). Beta-sitosterol belongs to the hydrophobic group of phytosterols with a chemical structure similar to cholesterols. It can be found in *Uncaria tormentosa*, *Aloe vera*, avocados or brown rice (Arndt 2008). β-sitosterol is used for treatment or prevention of heart diseases (reduces cholesterol levels in the blood), cancer (colon), diseases of the skin, allergies, migraine headache, cold or flu (WebMD 2009). But especially is important for inhibiting of enzyme activity 5-alfa reductase. This enzyme activity has increased the level of dihydrotestosterone (DHT), the substance causing the degenerative changes in man's organism, especially prostate enlargement and consequently benign prostate hypertrophy, degeneration of hair follicles (increased hair loss), increased fat storage and weakening of the immune system. Beta-sitosterol has antioxidant and anti-inflammatory potential too (Arndt 2008).

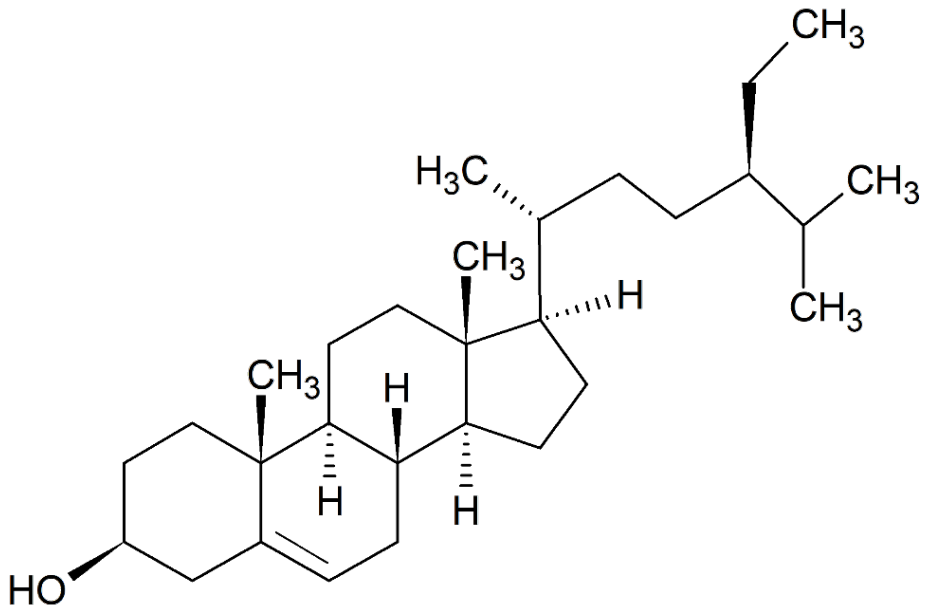

Figure 9 Beta-sitosterol

$C_{29}H_{48}O$ also known as stigmasterol is another representative of a group of phytosterols (National Center for Biotechnology Information 2018), contained in *Foeniculum vulgare* (Arndt 2008), plants or oils of soybeans, calabar beans, cocoa and rape seeds (Chemicalland21 2013). Several studies, for example, Synthesis of vitamin D3 and related compounds by Kametani and Furuyama (1987) proved his antioxidant potential and ability to the prevention of cancer, hypertension, reduces the level of cholesterol in the blood. Stigmasterol is used in medicine as starting material for the

production of synthetic progesterone (a female sex hormone important in the physiological role during the luteal phase of menstruation cycle) (Chemicalland21 2013).

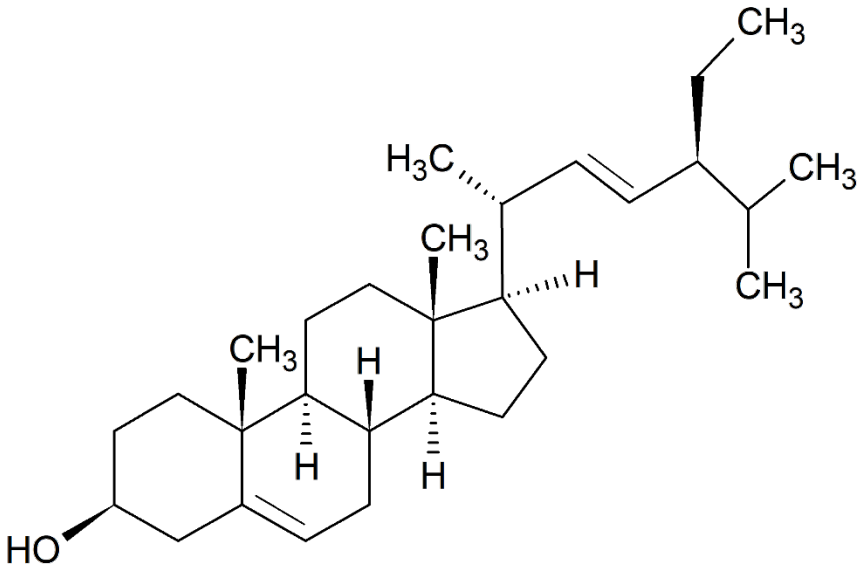

Figure 10 Stigmasterol

$C_{35}H_{60}O_6$ is a chemical formula of β-sitosterol 3-O-β-glucoside belongs to steroidal glycoside, the substances use against of heart diseases as heart failure or cardiac arrhythmia. The β-sitosterol 3-O-β-glucoside can be found in *Allium cepa* but the steroidal glycosides are contained in *Digitalis* genus, *Acocanthera ouabaio* or *Convallaria majalis* (Mizushina et al. 2006).

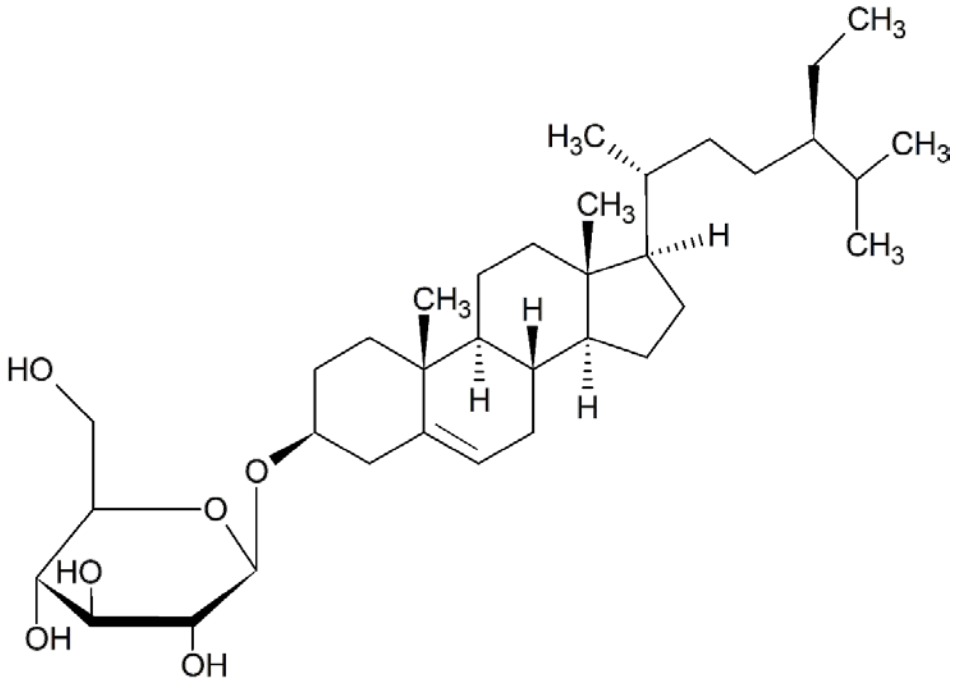

Figure 11 β-sitosterol 3-O-β-glucoside

Kaempferol 3-methyl ether 7-O-β-glucoside is chemical compounds belongs to glucoside of kaempferol – flavonoids. The general structure of flavonoids is characterized by a skeleton of 15 carbons, phenolic structure (two phenyl rings) and one heterocyclic ring (Calderón-Montaño et al. 2011). Flavonoids can commonly find in fruit and vegetables (Mindell 1997). Especially kaempferol are contained in vegetables and fruits with yellow color – grapes, broccoli, apples, potatoes, peaches and so on. And as other flavonoids can kaempferol regulating the cell cycle and metastasis in various cancer, especially ovarian cancer (Kim & Choi 2013).

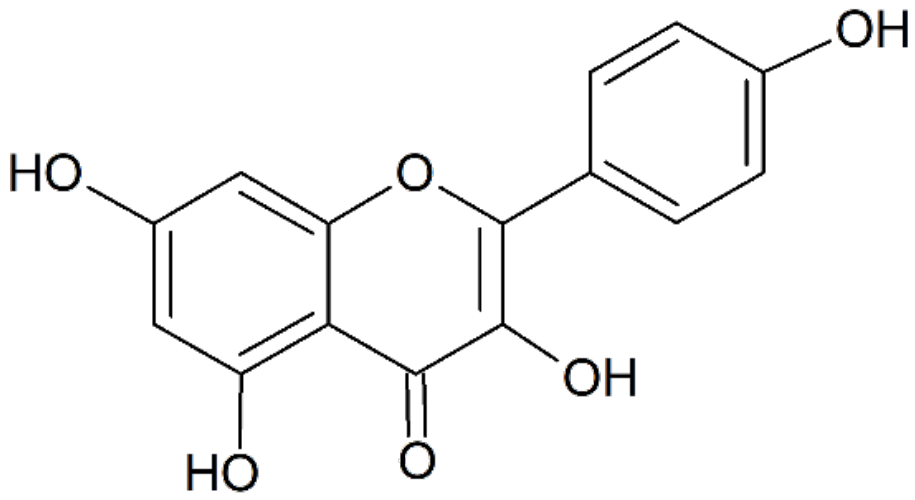

Figure 12 Kaempferol molecule

Apigenin 7-O-β-glucoside is composited of apigenin and glucoside. Apigenin belongs to the group of flavonoid and it is commonly contained in parsley, celery, celeriac and *Matricaria chamomilla.* From medicinal uses reports apigenin anti-proliferative activity against tumor cells and also anti-inflammation antioxidant potential. Also, it can help in brain diseases, disorders and injuries due to stimulating the neuronal brain cells (MedChemExpress 2015).

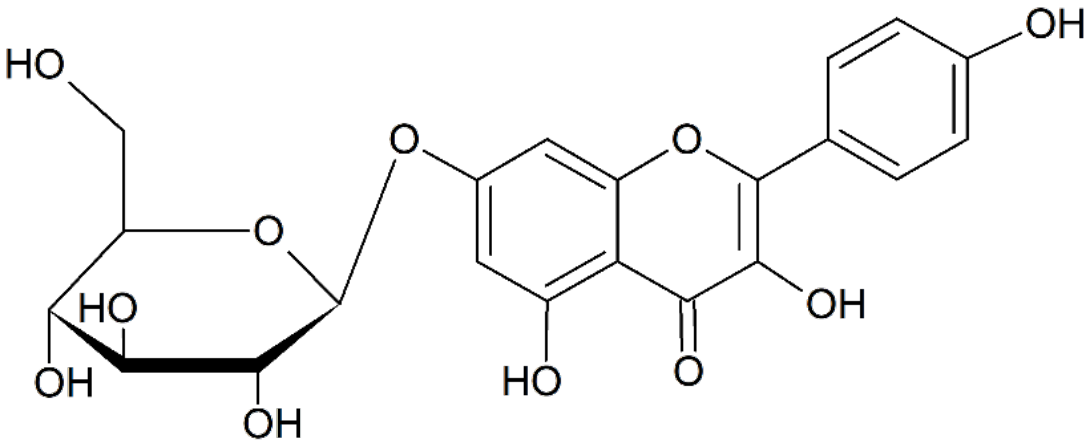

Figure 13 Apigenin 7-O-β-glucoside

Chemicals are accumulated mainly in leaves and stems. As other chemical compounds (Table 4) are contained crude protein (30.8%), dry matter (13.4%), minerals (Cu, Fe, Ca, Mg) and amino acids (threonine, lysine, and methionine) (Dieu et al. 2006).

Table 4 Chemical compounds in *Pseuderanthemum palatiferum* leaves (Dieu et al. 2006)

Minerals	mg/100 g fresh leaves	Amino acids	mg/100 g fresh leaves
Ca	875.50	Lysine	30.6
Mg	837.60	Methionine	29.7
Fe	38.80	Threonine	61.0
Cu	0.43	x	x

Amino acids are organic substances with a structure of carboxyl (-COOH) and amine (-NH2) functional group. They are used as building blocks of proteins. It exists twenty-two acids. Fourteen of them can human body create. Eight of them must human acquire in food or as the dietary supplement (Mindell 1997). Threonine, lysine, and methionine contained in *Pseuderanthemum palatiferum* are three amino acids like this. They are called essential amino acids and the human body cannot produce them (Laurichesse et al. 1998).

Threonine is the amino acid occurred in L-form, active form, in milk, gelatin, eggs and so on. It is important for the human nervous system, for porphyrin synthesis, fat metabolism and it prevents fat accumulation in the liver (PubChem 2018a).

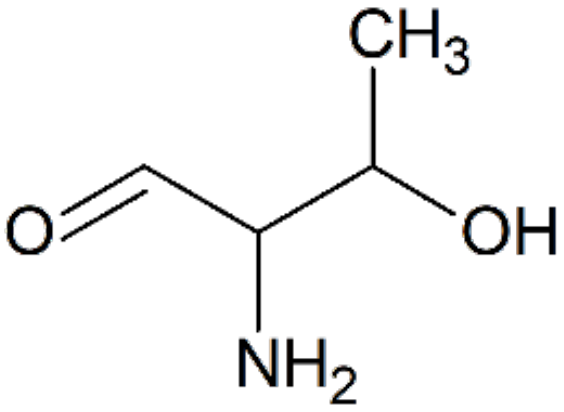

Figure 14 Amino acid threonine

Lysine is the amino acid which is help grow and repair of human tissue. It is contained in many foods (as fish, red meat and so on) and dietary supplements (PubChem 2018b).

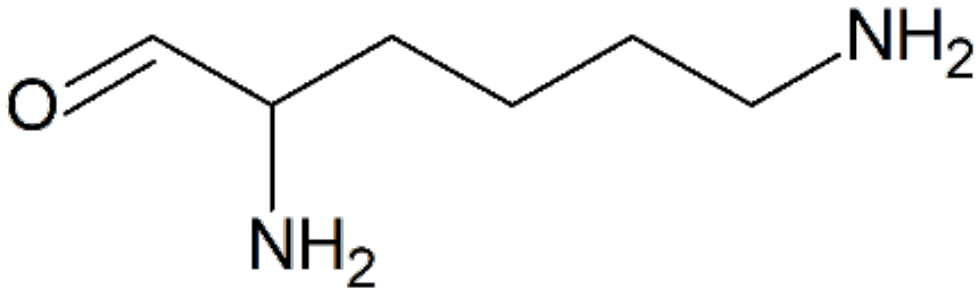

Figure 15 Amino acid lysine

Methionine is the amino acid with sulfur. Contained sulfur is important for pliability and tone of skin, nails, and hair, also for detoxifying process and slow cell aging. Without methionine could stop adsorption of zinc and selenium and chelating the heavy metals in the human body (PubChem 2018c).

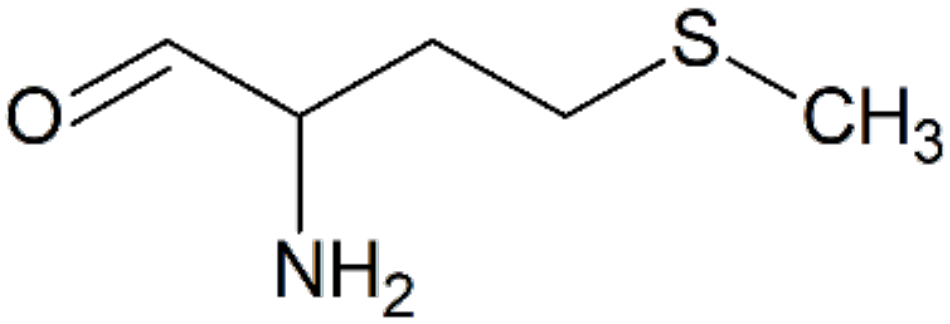

Figure 16 Amino acid methionine

III. Possibilities of using *Pseuderanthemum palatiferum*

Asian, especially Vietnamese, people consider this plant as a medicinal miracle (Dieu et al. 2006). According to the study of Dieu, Loc, Yamasaki, and Hirata (2005) people in fifteen villages in Cantho city (the center of Mekong Delta in Vietnam) cultivate *Pseuderanthemum palatiferum* in their gardens or houses. And consequently, they are chewing fresh leaves for treating diseases (65.9 %) listed in Table 5. The lower percentage of individuals use fresh leaves for prevention (21.5 %) against diseases listed also in Table 5. The number of dosed leaves is different for treatment or prevention of each disease and it also depends on individuals. For prevention is the most often used a quantity of 3 to 6 leaves per day (51.7 %), 7 to 9 leaves per day use 38 % and over 9 leaves use no more than 10.3 %. In case of treating disease is the situation a little different – 7 to 9 leaves per day use 80.9 % people.

Table 5 Leaves per day used for treatment of human diseases (Dieu et al. 2005)

Disease	**Leaves per day**
Blood urine	7-9
Blood vessel disease	7
Diabetes	7-9
Diarrhea	7-9
Dyspepsia	7-9
Heart disease	4-10
Hemorrhoids	7-9
High blood pressure	10
Chronic constipation	7
Inflammation of intestines	9
Inflammation of kidney	7-9
Inflammation of liver	7
Inflammation of stomach	9
Lymph cancer	10-20
Migraine headache	7-10
Muscle pain	7-9
Pink eyes	10
Rheumatoid	7
Skin disease	2-20
Uterus pain	7

Fresh leaves are not limited only to people. The study of Dieu at al. (2005) showed that dose of leaves can help animals, too. The most common treating diseases, listed in Table 6, are diarrhea or cholera. But the percent of animals use the fresh leaves is very low. For treatment take leaves 7.4 % animals and for prevention of diseases is it 3.7 % (Dieu et al. 2006).

Table 6 Leaves per day used for treatment of animal diseases (Dieu et al. 2005)

Animal	Disease	Leaves per day
Pig	Diarrhea	2-4
Dog	Diarrhea	1
Dog	Blood diarrhea	2
Chicken	Wound	1-3
Chicken	Fowl cholera	2-6
Duck	Fowl cholera	2

With increasing popularity of *Pseuderanthemum palatiferum* are traders from Vietnam and Thailand starting to produce commercial products from leaves. In these countries is selling herbal tea bags, capsules, powder decoctions, skin care products as shampoo, soap or lotions (Việc buôn bán, 2017).

Figure 17 Capsule extract of leaves and roots of *Pseuderanthemum palatiferum* (Việc buôn bán 2017)

Another option how to use this medicinal plant is a form of leaf extract. For research is obviously used a solution of ethanolic extracts (Chayarop et al. 2011). Dry sample from leaves is most often macerating with ethanol, acetone, methanol, and water. From these extracts are detected secondary metabolites of flavonoids, phenol carboxylic acids, and terpenoids and proved antioxidant potential and anti-inflammatory effects (Dieu et al. 2006).

Different possibility how to use *Pseuderanthemum palatiferum* is in the form of an essential oil with other plants reporting the medicinal effects. According to the study of Pamok et al. (2012) is consumption of *Moringa oleifera* and *Pseuderanthemum palatiferum* kind of prevention against colon cancer. The hypothesis of collaboration these medicinal plants are tested on three colon cancer cell line types HCT15, SW48 and SW480 with different solution (water and ethanolic) extracts from PP and *Moringa oleifera*. The results showed that the higher concentration of ethanolic plant extract from *M. oleifera* reports the antiproliferative effect on colon cancer cells, especially on SW48. Extract from *Pseuderanthemum palatiferum* is not so effective. But the reason for summary antiproliferative activity may be the antioxidative action of these two plants. Antioxidants contained in *M. oleifera* are vitamin C, α-tocopherol, flavonoids, phenolics and carotenoids (Makkar & Becker 1996) and in PP it is flavonoids, stigmasterol, salicylic acid and so on (Phan et al. 2003). It was proved the antiproliferative potential of the essential oil from *Pseuderanthemum palatiferum* and *Moringa oleifera* and it might be the new possibility of naturally therapeutic products against colon cancer (Pamok et al. 2012).

f. Analyses for detection the secondary metabolites contained in *Pseuderanthemum palatiferum*

I. Material

A phytochemical constituent contained in *Pseuderanthemum palatiferum* are analyzed from air-dried leaves at temperature 40-50°C. Dried and consequently powdered plant material is extracted by hot 96% EtOH for eight hours under the reflux. After 8 hours are solution filtrated and then the leachate is extracted again with hot 70% EtOH under the reflux too. The resulting extract is diluted with water in ratio 1:1 and divided into the parts with added n-hexane, $CHCl_3$ and ethyl acetate (Phan et al. 2003).

For recognition of chemicals contained in the sample is necessary to use some of the analytical techniques. These methods are divided into modern instrumental and classic quantitative and qualitative chemical analysis.

II. Analytical methods

i. Chemical analysis

Chemical techniques are primarily direct methods use stoichiometric reaction. These methods are used for identifying many chemical compounds in the sample and they are easy and mainly reliable and not very difficult on equipment. Indirect methods are used for comparative measurement and calibration dependencies. Classic techniques are often called as wet methods because for recognition chemical compounds is largely needed a liquid sample. But it does not always apply. Several chemical methods require dry sample. Quantitative chemical techniques collect information about sample which can be measured and quantified. It is including changes in concentration, weight, volume, and so on. But qualitative methods unlike quantitative cannot be measured. It is register changes in color, smell or texture. The first most important activity in starting analysis is to correctly take the representative sample and consequently his quartering. The second most important activity is choosing the right method of analysis.

The analysis is often starting with the qualitative method. This technique is selected reaction with a change in color or formation of a solution and it is waged in test-tubes, drops or as a microscopic reaction on a microscope slide. The following

method is quantitative. Gravimetric (weighing the sample before and after transformation), volumetric (titration – observe the color or solution changes in the sample) or colorimetric (color changes) analysis is measuring a number of chemical compounds contained in the sample.

According to other aspects of selection, analytical methods can be divided into organic or inorganic methods, destructive or non-destructive methods, determination of macro or microelements, analysis of liquids, gases, solids, and so on (Bartoš et al. 2004).

ii. Instrumental analysis

Some of the instrumental methods are similar to physicochemical (electrochemical) analysis but the most of them are purely physics (optical methods). Recently was largely used in emission spectroscopy for direct qualitative detection of solid samples and for a possibility of detection semi-quantitative estimation of concentration the chemical molecules in the sample. Currently, are more used in the spectral analysis in the visible part of spectrum (IR or UV) or X-rays (x-ray fluorescence or electron microscopy). The great benefit of instrumental analysis is the provision of qualitative and quantitative results (Bartoš et al. 2004). They are divided into the separative, optical, electrochemical, radiochemical and thermal analysis.

The separative method is characterized by dividing a sample at least in two parts with a different structure. The most used of separative analysis is chromatography. In this technique is the sample insert into the two immiscible phases. The first phase is named stationary and the second mobile. The sample is put into the beginning of the stationary phase. Due to a motion of the mobile phase across the stationary phase is the sample drifting through this system. Elements of the sample can be captured in stationary phase. This is the process of separation. There exist a lot of methods of separation and wherefore they are categorized according to form of mobile phase (liquid or gas chromatography), form of stationary phase (paper or thin layer chromatography) or form of predominate action (gel or separative chromatography).

Optical techniques are physical methods based on the interaction between the sample and electromagnetic radiation or on electromagnetic radiation from the sample.

They are divided into spectroscopic and non-spectroscopic methods. In non-spectroscopic methods are watched the property changes of radiance (change of velocity, plane rotation of polarized light or dispersion of radiance). In spectroscopic analysis is watched the energy exchange between solution and radiance and these methods are divided into emission (measuring the radiance from the sample) or adsorption (watching adsorption the radiance of the sample) analysis.

Electrochemical methods are techniques outgoing from the physical part of chemistry. The essential of these analyses is dependence research of electrochemistry behavior of concentration and composition in the sample. The object of research is an electrochemical system. In this system is the analyzed solution in contact with electrodes.

Radiochemical analyses have used a properties nuclei of the atom – radioactivity. Radioactive nuclei are unstable and it is changed into the nuclei of another nuclide. This transformation is connected with radioactive radiance. It can be distinguished α, β or γ radiance.

And finally, about thermic methods – these analyses are based on effects of supplied or non-supplied heat on the properties of the sample. Two most used methods are difference thermic analysis and thermogravimetric analysis (Klouda 2003).

For detection of secondary metabolites contained in *Pseuderanthemum palatiferum* was used several instrumental analyses, as IR (infrared) and UV (ultraviolet) from spectroscopic techniques, chromatography (over silica gel) and MS (mass spectrometry) from separated methods and H^1 NMR (hydrogen nuclear magnetic resonation) and C^{13} NMR (carbon nuclear magnetic resonation) from radioactive analyses (Phan et al. 2003).

a) Chromatography

The fraction with n-hexane is observed during the silica gel column chromatography. The reaction is using an n-hexane-EtOAc gradient for transforming into the solution with n-pentacosan-1-ol, stigmasterol, and β-sitosterol (Phan et al. 2003). The column chromatography over the silica gel is separated method with

stationary (the silica gel) and mobile phase (the extract from leaves of *Pseuderanthemum palatiferum*) (Clark 2007).

The extract with added chloroform is separated by MPL chromatography over the silica gel using different substances (chloroform, EtOAc, MeOH, H_2O) as eluents to give β-sitosterol 3-O-β-glucoside and stigmasterol 3-O-β-glucoside. MPLC is separated method using pumps for better extraction and consequently trapping the chemicals over the stationary phase (silica gel) from the sample (Bio-Rad Laboratories 2018).

And the last solution involving the extract of *Pseuderanthemum palatiferum* leaves and added EtOAc is chromatographed over silica gel together with chloroform, EtOAc, MeOH, H_2O to obtain kaempferol 3-methyl ether, 7-O-β-glucoside and apigenin 7-O-β-glucoside (Phan et al. 2003).

b) IR

Principe of infrared spectroscopy is adsorption of IR radiance in molecules. IR has a lower energy and higher wavelength (760 nm – 1 mm) than UV and VIS. Ultraviolet spectrum is therefore divided into three areas – close (0.78-2.5 μm), middle (2.50-50 μm) and far (50-1000 μm). In IR spectrometry is used instead of wavelength, repetency (reciprocal of the wavelength). The most important area of infrared spectrometry is 4000-670 cm^{-1}.

The energy of IR is not enough for electron changes. Therefore, the infrared adsorption specters are vibrational-rotatory. In IR specters are observed the dependence of transmittance or adsorption on repetency of adsorbing radiance. This specter has a form of a bond. The bonds in spectrum respond to different types of vibrant transition. About rotation – the molecule rotates around its center of gravity. The energy of rotation depends on the mass of bonding atoms and the length of the bond. The rotate transition can be measured in gas, but in liquid and solution can be detected. Unlike vibration – they can be measured in every form. The bond between atoms is characterized as spring. Vibration changing the length of the bond is called valence vibration (ν). Vibration changing the bonding angle is called deformation vibration. It is indicated with Greece letters according to vibration kind (Klouda 2003).

c) UV

The essence of UV and VIS is adsorption of the ultraviolet and visible spectrum (200-800 nm) in a diluted solution of molecules. In adsorption occurs to excitation of valence electrons which is the part of molecular orbitals (MO). MO are arising from nuclear orbitals (NO). From two NO are created two MO. One of them has lower energy than originated NO, called bonding MO, and second has higher energy than originated NO, called anti-bonding MO. Bonding electron pair is in the ground state in bonding MO. Into the anti-bonding MO can electron move during the adsorption of energy. This energy must correspond the difference of energies between both MO.

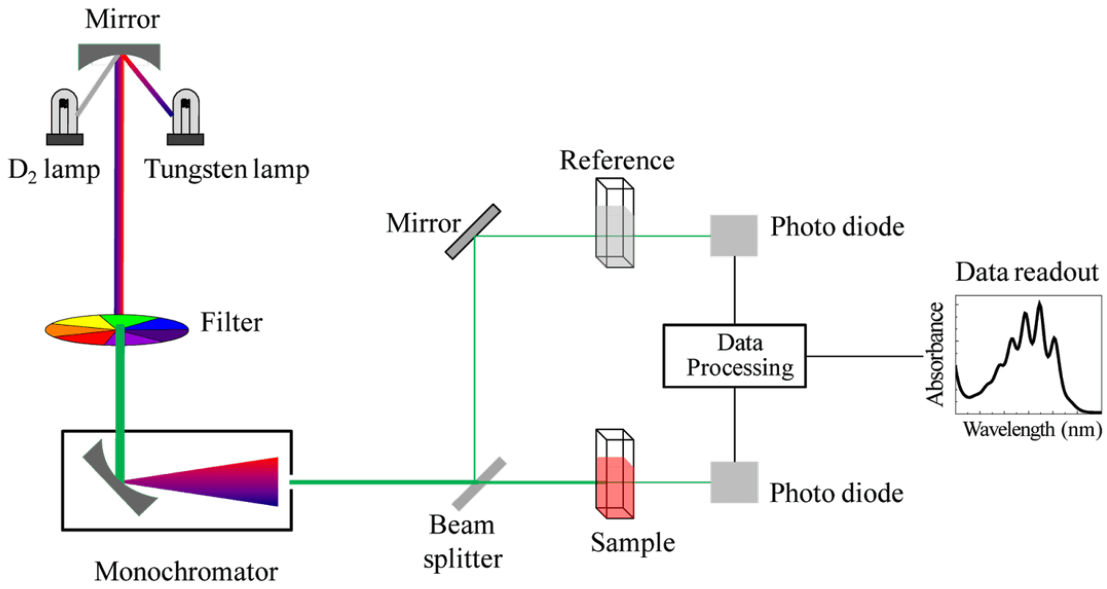

Figure 18 UV and VIS spectrometry (AAVOS International 2017)

d) MS

Mass spectrometry is separative technique transferring the sample into ionized gas form. Incurred ions are separated according to their weight and charge – m/s. The elemental steps in this methods are evaporation the sample, ionization, acceleration of ions into the mass analyzer, ions separation by the mass filter and finally detection of ions. As detectors of ions are most used Faraday cup (Klouda 2003).

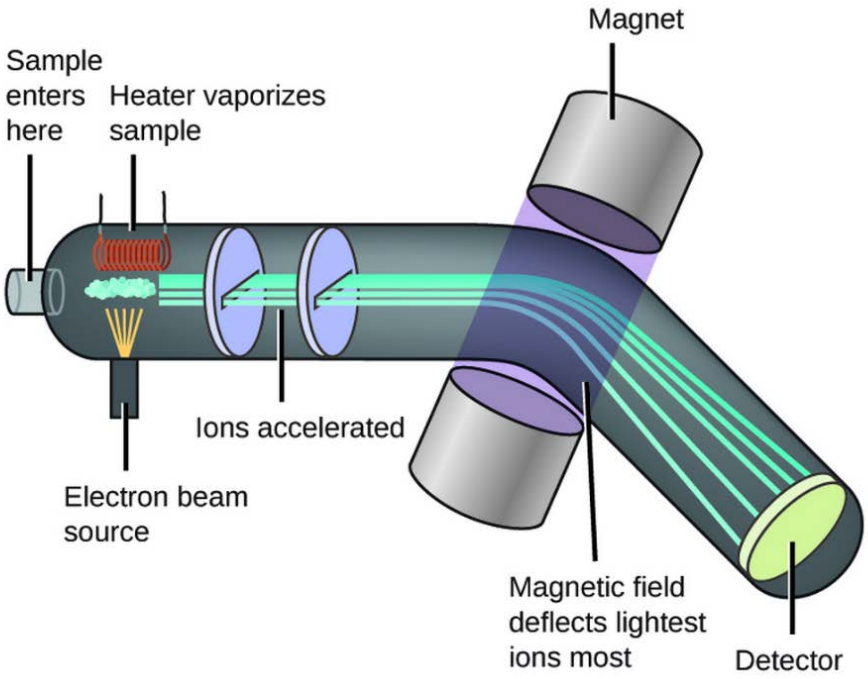

Figure 19 Mass spectrometer (Khan Academy 2018)

e) ^{1}H NMR and ^{13}C NMR

Nuclei of atoms of several elements have a magnetic moment. And into outside magnetic field are formed into the specific position of the energetic level. Adsorption of electromagnetic radiation in short wave attends to the transition of nuclei of atoms in higher energetic level (Klouda 2003).

Hydrogen nuclear magnetic resonance is the most important element for researching, but the interpretation of results is more difficult. In carbon NMR are the most focused results the position of carbons and how many carbons are in line (Ningh 2011).

NMR is used for identifying and structure analysis of organic chemicals, studies of chemical actions and balances in organic chemistry or for the study of hydrogen bonds. For unknown structures are using a library of specters for finding the elemental structure and then can be used for determination of molecular conformation in solution and simultaneously for physical properties on molecular level (Klouda 2003).

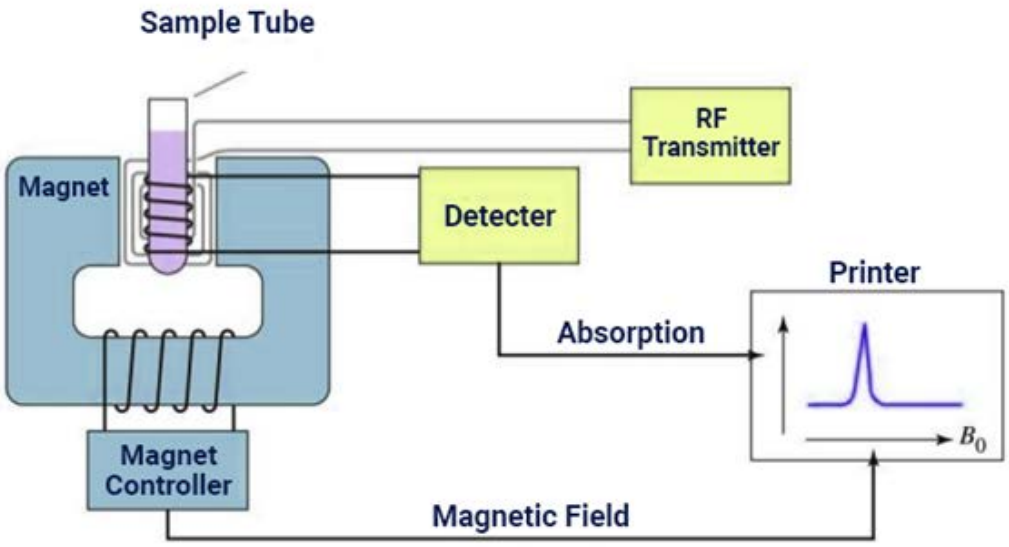

Figure 20 *The NMR spectroscopy* (BYJU'S CHEMISTRY 2017)

5. Conclusion

The first main objective of this literature review was summary of description and planting of *Pseuderanthemum palatiferum*, the new medicinal plant from Vietnam and family Acanthaceae.

The second main objective was observed the function and effects of secondary metabolites on human and animal health. According to several studies was proved that the substances contained in this medicinal plant could act as antioxidant and anti-inflammatory drugs. It can be used for treatment or as prevention against the lifestyle diseases in fresh form (chewing fresh leaves) or as the dietetic supplement (but these food supplements are not licensed yet).

Especially the pharmacological activity reports salicylic acid (treating diseases of skin), β-sitosterol (reduces cholesterol levels in the blood and thus prevent the risk of heart attack), stigmasterol (prevention of cardiovascular diseases) and kaempferol 3-methyl ether 7-O-β-glucoside and apigenin 7-O-β-glucoside (the antiproliferative effects on tumor cells).

6. References

21.století. 2010. Civilizační nemoci od A do Ž. Available from https://21stoleti.cz/2010/04/17/civilizacni-nemoci-od-a-do-z/ (accessed April 17, 2010).

AAVOS International. 2017. UV - VIS - spectrometry. Available from https://aavos.eu/glossary/uv-vis-spectrometry/.

Advanced Chemistry Development Inc. (n.d.). ACD/ChemSketch Freeware. Available from www.acdlabs.com.

Arndt T. 2008. Sitosterol. Available from https://www.celostnimedicina.cz/sitosterol.htm.

Axe D. 2018. Top 15 Anti-Inflammatory Foods. Available from https://draxe.com/anti-inflammatory-foods/.

Bartoš M, Šrámková J, Staněk V, Renger F, Kalous J. 2004. Analytická chemie I. Univerzita Pardubice, Fakulta chemicko-technologická, katedra analytické chemie, Pardubice. Available from https://meloun.upce.cz/docs/analchem1/skripta.pdf.

Bio-RadLaboratories. 2018. Introduction to Medium-Pressure Chromatography. Available from http://www.bio-rad.com/en-cz/applications-technologies/introduction-medium-pressure-chromatography?ID=MWHBD3HYP.

BYJU'S CHEMISTRY. 2017. NMR Spectroscopy. Available from https://byjus.com/chemistry/nmr-spectroscopy/ (accessed October 11, 2017).

Calderón-Montaño JM, Burgos-Morón E, Pérez-Guerrero C, López-Lázaro M. 2011. A Review on the Dietary Flavonoid Kaempferol. Mini-Reviews in Medicinal Chemistry **11**:298–344.

Clark J. 2007. COLUMN CHROMATOGRAPHY. Available from https://www.chemguide.co.uk/analysis/chromatography/column.html.

Debergh PC, Read PE. 1991. Micropropagation. Pages 1–13in P. C. Debergh and R. H. Zimmerman, editors.Micropropagation: Technology and Application. Springer Netherlands, Dordrecht. Available from https://doi.org/10.1007/978-94-009-2075-0_1.

Dieu H, Loc C, Yamasaki S, Hirata Y. 2005. The ethnobotanical and botanical study on Pseuderanthemum palatiferum as a new medicinal plant in the Mekong Delta of Vietnam. JARQ-JAPAN AGRICULTURAL RESEARCH QUARTERLY **39**:191–196. Available from https://www.jstage.jst.go.jp/article/jarq/39/3/39_191/_pdf.

Dieu H, Loc C, Yamasaki S, Hirata Y. 2006. The effects of Pseuderanthemum palatiferum, a new medicinal plant, on growth performances and diarrhea of piglets. JARQ-JAPAN AGRICULTURAL RESEARCH QUARTERLY **40**:85–91. Available from https://www.jstage.jst.go.jp/article/jarq/40/1/40_85/_pdf.

DRUGBANK. 2005. Salicylic acid. Available from https://www.drugbank.ca/drugs/DB00936 (accessed June 5, 2005).

Fitch WH. 1872. Eranthemum palatiferum Hook.f. Curtis's Botanical Magazine **28**:5963–6008.

Grulich V. 2016. ACANTHACEAE Juss. – paznehtníkovité. Available from http://botany.cz/cs/acanthaceae/ (accessed February 5, 2018).

Chayarop K, Peungvicha P, Wongkrajang Y, Chuakul W, Amnuoypol S, Temsiririrkkul R. 2011. Pharmaconostic and phytochemical investigations of Pseuderanthemum palatiterum (Nees) Radlk. ex Lindau leaves. Pharmacognosy Journal **3**:18–26. Available from http://eds.a.ebscohost.com.infozdroje.czu.cz/eds/pdfviewer/pdfviewer?sid=88d39210-081d-467a-a680-d0c3495bfefc%40sessionmgr4010&vid=1&hid=4213.

Chemicalland21. 2013. STIGMASTEROL. Available from http://chemicalland21.com/lifescience/phar/STIGMASTEROL.htm.

iGrandtour Corporation. 2012. Cuc Phuong National Park. Available from http://vietnamnationalpark.org/2660/cuc-phuong-national-park/ (accessed March 5, 2012).

Jones SB, Luchsinger AE. 1986. Introduction to Plant Systematics. McGraw-Hill College, McGraw-Hill College.

Kametani T, Furuyama H. 1987. Synthesis of vitamin D3 and related compounds. Medicinal Research Reviews **7**:147–171. Available from https://onlinelibrary.wiley.com/doi/abs/10.1002/med.2610070202.

Khan Academy. 2018. Isotopes and mass spectrometry. Available from https://www.khanacademy.org/science/chemistry/atomic-structure-and-properties/mass-spectrometry/a/isotopes-and-mass-spectrometry.

Khumpook T, Chomdej S, Saenphet S, Amornlerdpison D, Saenphet K. 2013. Anti-inflammatory Activity of Ethanol Extract from the Leaves of Pseuderanthemum palatiferum (Nees) Radlk. CHIANG MAI JOURNAL OF SCIENCE **40**:321–331. Available from https://www.researchgate.net/profile/Kanokporn_Saenphet/publication/253651912_Anti-inflammatory_Activity_of_Ethanol_Extract_from_the_Leaves_of_Pseuderanthemum_palatiferum_(Nees)_Radlk/links/0c96051fa895164249000000.pdf.

Kim S-H, Choi K-C. 2013. Anti-cancer Effect and Underlying Mechanism(s) of Kaempferol, a Phytoestrogen, on the Regulation of Apoptosis in Diverse Cancer Cell Models. Toxicological Research **29**:229–234. The Korean Society of Toxicology. Available from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3936174/.

Klouda P. 2003. Moderní analytické metody, 2nd edition. Nakladatelství Pavel Klouda, Ostava.

Krejčí P. 2008. Multimediální učební text obecná botanika. Available from http://web2.mendelu.cz/af_211_multitext/obecna_botanika/obrazky/organologie/postaveni_listu.gif (accessed May 28, 2008).

Kunte L, Zelený V. 2009. Okrasné rostliny tropů a subtropů. Grada Publishing a.s., Praha.

Lambers H. 2017. Plant reproductive system. Available from https://www.britannica.com/science/plant-reproductive-system (accessed November 17, 2017).

Laurichesse H et al. 1998. Threonine and methionine are limiting amino acids for protein synthesis in patients with AIDS. The Journal of nutrition **128**:1342–1348. United States.

Leech J. 2015. Phytosterols - "Heart-Healthy" Nutrients That Can Harm You. Available from https://www.healthline.com/nutrition/phytosterols-good-or-bad.

Lehman S. 2016. What is Phenolic Acid? Available from https://www.verywellfit.com/what-is-phenolic-acid-2507071.

MAHALO.cz. 2017. Fauna a flóra Vietnamu. Available from http://www.mahalo.cz/vietnam/informace-o-vietnamu/fauna-a-flora-vietnamu.html (accessed August 8, 2017).

Makkar H, Becker K. 1996. Nutritional value and antinutritional components of whole and ethanol extracted Moringa oleifera leaves. Anim. Feed Sci. Tech **63**:211–228.

Mateljan G. 2018. Flavonoids. Available from http://www.whfoods.com/genpage.php?tname=nutrient&dbid=119.

MedChemExpress. 2015. Apigenin 7-glucoside. Available from https://www.medchemexpress.com/Apigenin-7-glucoside.html.

Mindell E. 1997. Tajemná léčiva. Alpress, s. r. o., Frýdek-Místek.

Mizushina Y et al. 2006. β-Sitosterol-3-O-β-d-glucopyranoside: A eukaryotic DNA polymerase λ inhibitor. The Journal of Steroid Biochemistry and Molecular Biology **99**:100–107. Available from http://www.sciencedirect.com/science/article/pii/S0960076006000719.

National Center for Biotechnology Information. 2018. Stigmasterol. Available from https://pubchem.ncbi.nlm.nih.gov/compound/Stigmasterin#section=Top (accessed April 2, 2018).

Nguyenvan L. 2016. Cay hoan ngoc. Available from https://www.youtube.com/watch?v=SZGeiobASeo.

Ningh Y-C. 2011. Interpretation of Organic Spectra. John Wiley and Sons Ltd., Hoboken, United States.

Opletal L. 2016. Přírodní látky a jejich biologická aktivita. Nakladatelství Karolinum, Praha.

Ördög V, Molnár Z. 2011. Plant physiology. Debreceni Egyetem, Nyugat-Magyarországi Egyetem, Pannon Egyetem. Available from https://www.coursehero.com/file/11235172/0010-1A-Book-angol-01-novenyelettan-1/.

Pamok S, Saenphet S, Vinitketkumnuen U, Saenphet K. 2012. Antiproliferative effect of Moringa oleifera Lam. and Pseuderanthemum palatiferum (Nees) Radlk extracts on the colon cancer cells. Journal of Medicinal Plants Research **6**:139–145. Available from http://www.academicjournals.org/journal/JMPR/article-full-text-pdf/C98F2B816943.

Phan, M. G., Ha, V. B., and Phan TS. 2003. Phytochemical study on Pseuderanthemum palatiferum (Nees) Radlk., Acanthaceae. Journal of Chemistry **41**:115–118.

PubChem. 2018a. Threonine. Available from https://pubchem.ncbi.nlm.nih.gov/compound/L-threonine#section=Top.

PubChem. 2018b. Lysine. Available from https://pubchem.ncbi.nlm.nih.gov/compound/5962.

PubChem. 2018c. Methionine. Available from https://pubchem.ncbi.nlm.nih.gov/compound/6137#section=Top.

Rachna C. 2017. Difference Between Primary Metabolites and Secondary Metabolites. Available from https://biodifferences.com/difference-between-primary-metabolites-and-secondary-metabolites.html#respond (accessed July 20, 2017).

Rupam B, Gunja A, Sujan G, Shruthi SD. 2012. A review on medicinal plants having Antioxidant potential. Journal of Pharmacy Research **5**:4278–4287. Available from https://www.researchgate.net/profile/Sujan_Ganapathy/publication/233950638_A_review_on_medicinal_plants_having_Antioxidant_potential/links/02e7e534773fa524a3000000/A-review-on-medicinal-plants-having-Antioxidant-potential.pdf.

Straub D. 2006. Cuc Phuong Location. Available from www.planiglobe.com (accessed July 7, 2006).

Svobodová V. 2014. BOTANY.cz. Available from http://botany.cz/cs/pseuderanthemum-carruthersii/ (accessed January 4, 2014).

The University of North Carolina at Chapel Hill. 2018. Section A. Structure and Specialized Characters: XI. Fruits. Available from http://www.ibiblio.org/botnet/test/6-09-3.html (accessed February 5, 2018).

The Vitaminbox. 2018. MEDICINAL PLANTS - HOAN NGOC (3 PLANTS). Available from https://www.thevitaminbox.com/Hoan-Ngoc-Plant-p/sm0003.htm.

TUYET D. 2010. Characteristics of Karst Ecosystems of Vietnam and Their Vulnerability to Human Impact. Acta Geologica Sinica - English Edition **75**:325–329. Blackwell Publishing Ltd. Available from http://doi.wiley.com/10.1111/j.1755-6724.2001.tb00539.x (accessed August 8, 2017).

United States Department of Agriculture: Natural Resources Conservation Service. 2017. Plant Profile: Pseuderanthemum Radlk. Available from https://plants.usda.gov/core/profile?symbol=PSEUD21 (accessed August 2, 2017).

Việc buôn bán. 2017. HOAN NGOC - TANU GREEN. Available from https://www.viecbuonban.com/functional-food/hoan-ngoc-tanu-green.

Watson L, Dallwitz MJ. 1992. The families of flowering plants : descriptions, illustrations, identification, and information retrieval. [publisher not identified], [Australia]. Available from http://delta-intkey.com/angio/.

WebMD. 2009. Find a Vitamin or Supplement (beta-sitosterol). Available from https://www.webmd.com/vitamins-supplements/ingredientmono-939-beta-sitosterol.aspx?activeingredientid=939&activeingredientname=beta-sitosterol.