VIETNAM NATIONAL UNIVERSITY, HANOI

VNU UNIVERSITY OF SCIENCE

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ETHNOBOTANICAL STUDY ON THE TRADITIONAL KNOWLEDGE OF MEDICINAL PLANTS IN BAC HUONG HOA NATURE RESERVE, QUANG TRI PROVINCE, VIETNAM

Branch: Botany
Code: 62422001

THE EXECUTIVE SUMMARY OF DISSERTATION
FOR THE DEGREE OF DOCTOR OF BOTANY

HANOI - 2022

The dissertation was performed at VNU University of Science

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INTRODUCTION

1. The importance of the topic

Plant resources have a long history of being used as medicinal materials. It is often cited that 80% of the world's population still relies on traditional medicines to meet their primary health care needs (WHO, 2008) and nearly 25% of modern medicines are derived from nature, many of which were derived from traditional uses (De et al., 2012). The use of traditional medicines is generally affected by accessibility, availability, and acceptability of health care services (Gaitonde and Kurup, 2005). Especially, in remote areas of developing countries, medicinal plants may form the only available source of health care (Van Andel, 2000).

Vietnam possesses a vast wealth of historical, cultural, and natural richness that includes big deltas, huge limestone towers, beautiful sand dunes, lush green forests and grass-lands. The richness can also be seen in the diversity of flora which is evident with an estimated number of 13,747 vascular plant species (MONRE, 2014). To preserve the rich biodiversity of Vietnam and their sustainable development, the government has demarcated 164 special use forest areas including 30 National Parks, 58 Natural Reserves, 11 Species Conservation Areas, 45 Forest Landscape Protection and 20 Experimental Forests of Scientific Research (MONRE, 2014). The use of traditional medicine in Vietnam has a vibrant history spanning over thousands of years. Today, around 75% of Vietnamese people use traditional medicine as their primary source of treatment to meet their health care needs. These users mostly include inhabitants of rural or mountainous areas which have less accessibility to hospitals or community health care centers (Van et al. 2008).

The Van Kieu ethnic minority is one of the 54 officially recognized ethnic minorities of Vietnam, whose population is around

74,500 as of 2009 (GSO, 2010), of which 55,079 (73.9%) live in Quang Tri province. They are largely residing around two protected areas, Dakrong and Bac Huong Hoa Nature Reserves (Birdlife International, 2013). The people belonging to the Van Kieu ethnic group are extremely poor and heavily depend on forest resources such as wood and non-timber forest products. Their major livelihood activities include collection of forest products such as wild honey, bamboo shoots, animals and mushrooms for consumption (Nguyen et al. 2015).

Contrary to the growing demand of medicinal plants all over the world, traditional knowledge is declining rapidly, especially in the developing countries (Hassan et al. 2005). Considering the vast floristic wealth of the Nature Reserve and the rich knowledge of the local community, an urgent need was felt to take over this work which could help the researchers, forest officials as well as the future generation people involved in ethno-botany research. It is worth mentioning that the knowledge of Van Kieu ethnic people about the medicinal plants is vast but poorly documented.

2. Objectives

The need for the conservation of ethnobotanical knowledge was the core motivation for the conduct of this research with the use of quantitative techniques. This method is the first ever study which documents the valuable ethnobotanical information on the medicinal plants used by local Van Kieu ethnic people of Bac Huong Hoa Nature Reserve of Vietnam.

Hence, the objectives of this thesis are as follows:

- Document the medicinal plants and their use in traditional knowledge
- Compare the generated data by this study with previously published Dictionary of Vietnam Medicinal plants (DVM)
- Provide information on the antioxidant, anti-inflammatory and

anticancer activity via thirty methanol extracts taken from BHHNR medicinal plants.

3. The new points of this dissertation

3.1. First-ever study to document the traditional medicinal knowledge of the Van Kieu ethnic people from Bac Huong Hoa Nature Reserve, Vietnam.

3.2. Eight medicinal plants used by Van Kieu ethnic people have not been previously reported in DVM.

3.3. Provide detailed information to illuminate the complete chloroplast genome structure of *D. tonkinensis* and clarified the phylogenetic relationships within Papilionoideae.

3.4. Adding new data on the potential treatment of anti cancer, anti in-flammation and anti oxidant of some species.

Chapter 1. LITERATURE REVIEW

1.1. Overview of Ethnobotany and Medicinal plant

1.1.1. Ethnobotany definition and history

Ethnobotany is part of the discipline ethnobiology, which studies the dynamic relationships among peoples, biota, and environments (Ethnobiology Working Group, 2003). The term ethnobotany was first suggested by John Harshberger in 1896 to delimit a specific field of botany and describe plant uses. It was defined as "the use of plants by aboriginal peoples" (Cotton, 1996). Worth noting is the concept of Mahishi et al. (Mahishi et al. 2005), who depicted ethnobotany as the renaissance of traditional herbal medicine. In that case, medicinal plants are botanical remedies derived from trees, shrubs, or herbaceous plants that are useful for primary healthcare system and as a remedy for disease and injury including plants used traditionally for foods and drinks that are also believed good for health (Dawit Abebe and Ahadu Ayehu, 1993). Medicinal plants play multipurpose roles such as spices and condiments (Jansen, 1981), apiculture (Fitchtl R. and Admsu Adi, 1994), ecological services, source of wood and wood products as well as soil conservation in addition to their medicinal value (Legesse 1995).

1.1.2. Medicinal plant

According to the World Health Organization (WHO, 1977) "a medicinal plant" is any plant, which in one or more of its organs contains substances that can be used for the therapeutic purposes or which, are precursors for the synthesis of useful drugs. This definition distinguishes those plants whose therapeutic properties and constituents have been established scientifically and plants that are regarded as medicinal, but which have not yet been subjected to thorough investigation. Medicinal plants are main ingredients of herbal products/traditional medicine. It is based on the hereditary experience and made of plants materials, animals or minerals, not in the form of pure substance. According to Setiawan, medicinal plant is considered as

parts of the plant such as leaves, stems or roots which have efficacy as a drug and is used as a raw material in the manufacture of modern either traditional medicine.

World Health Organization reported that medicinal plant has a promising future because there are about half million plants that already identified and classified in around the world. Most of them have medical activities which have not been investigated yet. Furthermore, their medical activities could be decisive in the treatment of present or future studies.

1.1.3. Medicinal plant in Vietnam

Traditional medicine in Vietnam dates back at least to the 2nd Century B.C. (Le Tran Duc, 1995). It has its foundations in the basis of the yin-yang principle and the interaction between human bodies and their surrounding environments. Traditional Vietnamese Medicine (TVM) remedies were documented for the first time in the ten-volume "The Miracle of Southern Medicine" written in the 14th Century by Tue Tinh, which listed approximately 500 naturally sourced materials forming the basis for over 3,800 traditional remedies, which were used to cure 182 diseases and ailments (Pham Xuan Sinh and Phung Hoa Binh, 2002).

During the French colonial period (19^{th} and early 20^{th} Centuries), French botanists continued the research into the taxonomy of Viet Nam's medicinal flora when they collected information for the book "General Flora of Indochina" (Flore Générale de L'Indochine) (Lecomte, 1907-1952). However, the French regime did not encourage the use of traditional medicine or include it in the formal healthcare system, so western medicine became dominant during this period (Pham Xuan Sinh and Phung Hoa Binh, 2002). Traditional medicine has, however, always remained very popular among Vietnamese people and today it is often used in parallel with, or in replacement of, western medicine.

Traditional medicine is typically perceived to produce few or no

side effects and is believed to be able to cure certain health problems that western medicine cannot. It is also believed that while bacteria and viruses can develop resistance to western medicines, there has been no similar finding from studies on traditional medicines (Le Dien Duc, 1994). Traditional medicines are characterized by the use of crude herbs and prolonged usage. A single herb may contain a great many natural constituents and a combination of herbs even more. Since the constituents in the plants work better in conjunction with each other, it is believed that the patient is able to achieve significant long-term benefits by using traditional medicine remedies (Zhang Xiaorui, 1998).

Today, an estimated 75% percent of Vietnamese people use traditional medicine as their primary source of treatment for common health problems (Nguyen Dao Ngoc Van and Nguyen Tap, 2008). This statistic is in keeping with the WHO, which has estimated that approximately 80% of the population in developing countries still depend on traditional medicines, particularly those made from plants (Shi-lin Chen and Christin Leon, 2006).

Ethnomedicinal plants studies in Vietnam:

Tran Thien An and Ziegler (2001) have documented 432 plant species from Bach Ma National Park with the mentions of their medicinal uses. Tran Van On et al., (2001) and Hoang Van Sam (2012), have documented the traditional knowledge on medicinal plants in Ba Vi National Park. Van Sam et al., (2008) have documented 230 species from Ben En National Park with their medicinal uses. Vo Van Minh et al. (2014), have documented 45 species in Ba To district area with the medicinal uses of indigenous people. Many researchers have studied ethnobotanical medicinal plants in Vietnam (Ogle et al., 2003), However, compared to the enormous medicinal flora in Vietnam still a small part of traditional ethnomedicinal knowledge has been recorded and documented

1.1.4. Van Kieu Ethnic group

In Central Vietnam, ethnic minorities live in the mountainous area on the eastern slope of the Annam Cordillera. Different from the lowland centers of population and politics in the eastern coast, the dominant geographic feature of this area is forest with lower population density. The focus of this study is the southernmost upland area of northern central Vietnam where until 1975 was still under contestation between opposing political entities in the Second Indochina War. During this period the main residents of this area were different ethnic minority groups in Mon-Khmer language family including the Pacoh, Van Kieu, Taoih and Katu. Because of the conflict no political entities were able to establish a functioning civilian governance system (Nguyen Trinh Minh Anh, 2016).

The Van Kieu ethnic minority is one of the 54 officially recognized ethnic minorities of Vietnam, whose population is around 74,500 as of 2009 (GSO, 2010), of which 55,079 (73.9%) live in Quang Tri province. They are largely residing around two protected areas, Dakrong and Bac Huong Hoa Nature Reserves (Birdlife International, 2013). Traditionally, shifting cultivation and collection of forest products were the main means of livelihood for the Van Kieu. In shifting cultivation, local people used technique that they summarized as "clearing forest, burning dried vegetation, making holes by pricking the ground with stick; and burying crop seeds in the holes" (phat, dot, cot, tria in Vietnamese) to plant local rice, corn, bean, and cassava (Nguyen et al. 2015). Meanwhile, collection of forest products such as wild animal, mushrooms, honey, bamboo shoots for household consumption was also a major livelihood activity, especially important in cases of crop failure. Forest and forest land were arguably the most important resources for sustaining the Van Kieu's subsistence economy. The governance of these resources was based on unwritten customary rules built around shared beliefs and experience. Village was the main social

unit of Van Kieu people (Nguyen et al. 2015).

There was limited encounter with people from outside village, either inter-ethnically or intra-ethnically (Hong, 2002). As a patriarchal society, social network was primarily kinship of paternal line where men made decision regarding activities related to the survival of the family and the community while women were in charge of household work, crop planting, weeding, animal feeding and harvesting forest minor products.

Under the Constitution of Vietnam all ethnic groups have equal social and political rights regardless of minority or dominant status. Ethnic minority groups play an important role in anti-colonial struggle and Second Indochina War. Since the foundation of modern state in Vietnam in 1945, ethnic minority groups are often encouraged to participate in political institutions. During the field survey, it was not uncommon to see Van Kieu or Pacoh individuals working in political and administrative institutions at commune and district level. The assignment of ethnic minority people to local political positions, however, is not a guarantee for preservation and exhibition of full range of social and cultural life of ethnic minority. The state views ethnic minorities as being at the early stage in Marxist-Leninist framework of social evolution in which development proceeds in stages from primitive to modern and socialist (McElwee, 2004).

This official view was resonated by various authors claiming that the Van Kieu's traditional farming system using simple tools such as a machete, ax and stick, the phat, dot, cot, tria technique and its low productivity were proof of their "backward-ness" while animistic beliefs in the existence of individual spirit in natural phenomena and entity such as river, mountain and forest was proof of their "superstitious-ness" (Hong, 2002).

1.3. Overview biologically activity of plants

Natural products, such as plants extract, either as pure compounds

or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity (Cosa et al., 2006). According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Duraipandiyan et al., 2006).

Chapter 2. MATERIALS AND METHODS

2.1. General description of the study area

2.1.1. Location

The study was conducted in Bac Huong Hoa Nature Reserve (BHHNR), located in Central Vietnam. The study area covers 25,200 hectares, including 20,646.2 hectares of natural forest and is located in the north of Huong Hoa District of Quang Tri Province, 50 km north-west of Khe San town, and 120 km west of Dong Hai town (16°43'22″ N to 16°59'55″ N latitude and 106°33'00″E to 106°47′03″ E longitude) (Figure 2.1).

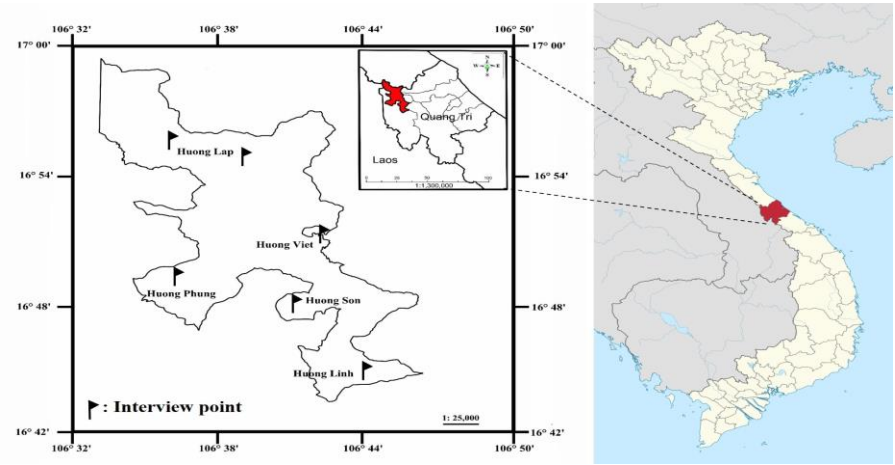

Figure 2.1. Location of the study area

2.2. Data collection

2.2.1. Informant sampling

The permission was obtained from Quang Tri Forest Protection Department (FPD), BHHNR to conduct our research (Permission No.: 857/UBND-NN, 230/SNgV-LSVK). The survey was conducted from April 2016 to March 2018. A total of 93 people were interviewed

belonging to Huong Linh, Huong Son, Huong Phung, Huong Viet and Huong Lap Communities (Figure 2.1).

2.2.2. In-depth semi-structured interview

Each participant was interviewed in isolation to avoid the possibility of one informant's answer influencing another's answer. The informants were asked about their personal experience or participation in the use and preparation of medicinal plants. At times, pictures were shown to informants to make sure that they could identify what plant was being asked about.

2.2.3. Plant collection and identification

The collected plants were identified using "An Illustrated Flora of Vietnam" (Ho, 2000) and DNA sequencing. Scientific names of the plant species were determined according to the Plant List (http://www.theplantlist.org) and voucher specimens were deposited at the herbarium of Korea Research Institute of Bioscience and Biotechnology (KRIB) in Republic of Korea and in the herbarium of Vietnam Academy of Science and Technology (HN) in Vietnam.

2.2.4. DNA extraction, PCR, and sequencing

Specimens which could not be morphologically identified due to unfamiliarity or lack of reproductive parts were determined molecularly by DNA sequencing in Korea, and by comparing the sequences with those found in the GenBank through BLAST search.

PCR amplification of the four fragments including ITS, *matK*, *trnH-psbA* and *rbcL* were selected. PCR reaction system was optimized and modified based on the recommended protocol (CBOL Plant Wording Group, 2009).

2.3. Data analysis

2.3.2. Establishing use categories

The ethnobotanical data were analyzed using Microsoft Office Excel Spreadsheet 2010. The categories suggested for diseases were classified according to International Classification of Primary Care (ICPC; http://www. who.int/classifications/icd/adaptations/icpc2/en/) system for intercultural comparisons (Soler et al. 2008; Staub et al. 2015).

2.3.3. Informant Consensus Factor (ICF)

Informant Consensus Factor (ICF) is used to check an agreement in the use of a plant species in particular ailment category by the users. It was calculated by using following formula.

ICF = (Nur − Nt)/(Nur − 1), where Nur is the number of use reports in a particular category and Nt is the number of plant species recorded in the category (Trotter and Logan, 1986).

2.4. Screening of ethnomedicinal plants for biological activities

2.4.1. Extraction

Dried plants were pulverized using a cutting mill, followed by extraction using methanol solvents.

2.4.2. Anticancer activity

To evaluate effects of extracts on the cell viability, a Cytotoxicity assay was performed. The cancer cells (AGS, A549, HCT116, MCF7, HepG2) were seeded onto 96-well plate at 1 x 105 cells/ml well then, cultured in 5% CO2 incubator at 37℃ for 24 hrs.

2.4.3. Anti-inflammation activity

Cells were cultured in DMEM supplemented with 5% of FBS (Fetal Bovine Serum) incubated 4hr at 37°C and 5% CO_2. RAW 264.7

macrophages were seeded in 96-well plates at density of 1×10^4 cells.

2.4.4. Cell viability

Cells were cultured in DMEM supplemented with 5% of FBS (Fetal Bovine Serum) incubated 4 hr at 37°C and 5% CO2. RAW 264.7 macrophages were seeded in 96-well plates at density of 1×10^5 cells/well and 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay was performed to measure the cell viability.

2.4.5. DPPH radical scavenging activity

The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable DPPH free radical according to the method described by Brand-Williams et al. (1995) with slight modifications.

Chapter 3. RESULTS AND DISCUSSION

3.1. Traditional Knowledge of medicinal plants in BHHNR

3.1.1. Demographic characteristics of informants

Out of 93 people interviewed, 51 informants were men (54.8%) while 42 were women. The age of the informants varied from 20 to 81 and the mean age was 45. The majority of the local Van Kieu ethnic people interviewed were elementary (91.4%), 7.5% with secondary education and 1.1% with higher-secondary education (Table 3.1).

Table 3.1. Demographic characteristics of informants

Demographic characteristics	Number (%)
Age	
20-40	29 (31.2)
41-50	27 (29.0)
51-70	28 (30.1)
71 and above	9 (9.7)
Gender	
Male	51 (54.8)
Femal	42 (45.2)
Educational level	
Elementary	85 (91.4)
Secondary	7 (7.5)
Higher-Secondary	1 (1.1)

3.1.2. Plant taxa identified by DNA barcoding

Difficulties in morphological identification among the collected plants, were identified and determined by DNA sequencing (ITS, *trnH-psbA*, *rbcL* and *matK*) and by comparing the sequences with those present in the GenBank.

3.1.3. General data on medicinal species

A total of 112 medicinal plant species belonging to 102 genera of 46 families were recorded from our investigation area, BHHNR. The plant species recorded in the study area are presented in Table 3.3,

arranged in an alphabetic order for families and entities. The most frequently used families were Euphorbiaceae (10 species), Compositae and Leguminosae (9 species each), Apocynaceae (7 species), Rutaceae and Rubiaceae (5 species each). The remaining families are represented by 4 or fewer entities. These families have been widely used in the Indo-Burma region for their medicinal properties (Phumthum et al., 2018).

3.1.4. Plants parts used and route of administration

Leaves represent the most used plant part (43.1%), followed by roots (24.4%), stems (13.1%), whole plants (6.3%), fruits (5.0%), latex and barks (3.1%) and seeds (1.9%).

3.1.5. Preparation and administration

Seven different modes of medicine preparation were documented. Decoction (49%) was the most frequently quoted mode of preparation (Figure 3-3), followed by crushing (22.8%), juice (13.5%), raw (5.6%), alcohol maceration (4.8%), burn (3.9%) and cook with pork (0.3%).

Total 9 routes of administration were quoted by Van Kieu people (Figure 3-4). Oral administration was the most frequently used route (51%), followed by Application to skin (12.1%), Sauna (11.3%), Fomentation (9%), Chew (5.4%), Application to injury places (4.5%), Bath (2.8%), Dye teeth (2.5%) and Fumigation (1.4%).

3.1.6. Combination of medicinal plants

Van Kieu ethnic people combine some species to treat few diseases (Table 3.4). For example, a decoction of *Mussaenda hoaensis* Pierre ex Pit. (Stem and leaves) along with *Mimosa pudica* L. (whole plant), *Smilax corbularia* Kunth (roots) and *Centella asiatica* (L.) Urb. (whole plant) is used to treat uterus fibromyoma and gonorrhoea in females. Similarly, a decoction of *Cryptolepis dubia* (Burm.f.) M.R. Almeida (roots) and *Mussaenda hoaensis* Pierre ex Pit. (roots) is used to treat vaginitis.

Table 3.4. List of plant combinations used by Van Kieu ethnic people

Scientific name (parts used)	Preparation method	Medicinal use
Aquilaria crassna (R) + *Artocarpus heterophyllus* (L) + *Citrus* × *aurantium* (R)	decoction / oral	Fever, Abdominal pain
Litsea cubeba (S, L) + *Citrus maxima* (L) + *Mangifera indica* (L) + *Artocarpus heterophyllus* (L)	decoction / Sauna	Post-partum symptom
Wendlandia uvariifolia subsp. *laotica* (L) + *Citrus maxima* (L) + *Cymbopogon flexuosus* (S) + *Citrus* × *aurantium* (L)	decoction / sauna	Health maintenance
Polygonum odoratum (R) + *Citrus* × *aurantium* (R)	alcohol maceration /chew	Teeth/gum disease
Lantana camara (R) + *Mangifera indica* (R)	decoction / oral	Abdominal pain
Lantana camara (R) + *Mangifera indica* (R) + *Erythrina variegata* (B)	decoction / oral	Diarrhoea
Cryptolepis dubia (R) + *Mussaenda hoaensis* (R)	decoction / oral	Vaginitis
Melia azedarach (L) + *Jatropha curcas* (L)	Crushed mix with salt / apply to skin	Acne
Blumea balsamifer (L) + *Chromolaena odorata* (L)	Decoction /bath	Menstruation irregular, Genital disease female
Sida rhombifolia (R) + *Mimosa pudica* (R) + *Elephantopus scaber* (R)	alcohol maceration / apply to skin	Lump/swelling localized
Litsea cubeba (L) + *Phoebe tavoyana* (S, L) + *Cinnamomum balansae* (S, L)	decoction / Sauna	Post-partum symptom
Mussaenda hoaensis (S, L) + *Mimosa pudica*(W) + *Smilax corbularia*(R) + *Centella asiatica*(W)	decoction / oral	Fibromyoma uterus, Gonorrhoea female
Averrhoa carambola(L) + *Eclipta* prostrata (W)	decoction / oral	Fever

W= whole plant; L= leaves; R= roots; B= bark; S= stems

3.2. Data Analysis

3.2.1. Use-Reports (UR)

Total 356 use-reports were documented in this study. The species with the high use-reports (UR) were *Artocarpus heterophyllus* Lam. (14 UR), *Chromolaena odorata* (L.) R.M.King & H.Rob. (13 UR), *Blumea balsamifera* (L.) DC., *Psidium guajava* L. and *Catunaregam spinosa* (Thunb.) Tirveng. (9 UR), *Mussaenda hoaensis* Pierre ex Pit., *Citrus* × *aurantium* L. and *Jatropha curcas* L. (8 UR).

3.2.2. Informant Consensus Factor (ICF)

The ailments reported by informants were grouped into 14 categories (Table 3.5). The highest values of Informant Consensus Factor (ICF) were recorded for Eye diseases (ICF=1.0, included eye injuries and other), followed by Musculoskeletal disorders (ICF =0.76, included musculoskeletal and rheumatic disorders. Eye diseases, endocrine/metabolic and nutritional disorders category had less UR and plant species, so it was exhibited high degree of consensus with ICF values. The highest UR and plant taxa used were recorded for digestive problems (which included abdominal pain, teeth/gum diseases, diarrhoea etc.).

Table 3.5. Informant consensus factor for commonly used medicinal plants

No.	Category	Number of taxa (Nt)	Use reports (Nur)	ICF
1	EY=Eye	1	2	1.00
2	MU=Musculoskeletal	9	35	0.76
3	EN=Endocrine/Metabolic and Nutritional	2	4	0.67
4	PR=Pregnancy, Childbearing, Family Planning	12	32	0.65
5	FE=Female Genital	7	17	0.63
6	UR=Urological,	4	9	0.63
7	GE=General and Unspecified	28	70	0.61
8	SK=Skin	24	58	0.61
9	RE=Respiratory,	7	16	0.60
10	DI=Digestive	43	94	0.55
11	CA=Cardiovascular,	6	11	0.50
12	NE=Neurological,	2	3	0.50
13	PS=Psychological,	3	4	0.33
14	B=Blood, Blood Forming Organs and Immune Mechanism	1	1	0.00

3.2.3. Rare plants in IUCN Red List

Out of 111 plants studied from this region, three plant species have been listed in the IUCN Red list (IUCN, 2018) viz. *Dalbergia tonkinensis* Prain as Vulnerable (VU), *Cinnamomum balansae* Lecomte as Endangered (EN) and *Aquilaria crassna* Pierre ex Lecomte as Critically Endangered (CR).

3.2.4. Complete Chloroplast genome sequencing of D. tonkinensis

The complete chloroplast genome of *D. tonkinensis* has a total length of 156,086 bp, with a pair of inverted repeats (IRs) of 25,720 bp that separate a large single copy (LSC) region of 85,761 bp and a small single copy (SSC) region of 18,885 bp. This study has provided detailed information to illuminate the complete chloroplast genome structure of *D. tonkinensis,* and clarified the phylogenetic relationships within Papilionoideae. And it is expected to provide fundamental potential information for effective conservation as well as management of the vulnerable (VU) plant species.

3.2.5. Comparison to Dictionary of Vietnam Medicinal Plants (DVM)

In 2012, Dictionary of Vietnam Medicinal Plants (DVM) was published comprising about 3,200 medicinal plants from Vietnam (Vo Van Chi, 2012). It is the most widely used book in Vietnam and contains information about the traditional and modern medicine. 62 (55.86%) species from this study were reported for the similar use as in DVM while 41 species (36.94%) were reported to have different uses than that of DVM.

3.3. Screening of ethnomedicinal plants for biological activities

3.3.1. Screening of ethnomedicinal plants for anti-inflammatory activity

Inflammatory is a tissue response to harmful stimuli, such as pathogens and damaged cells or irritants thereby, inheriting to the pathogenesis of a variety of diseases.

3.3.1.1. Screening of ethnomedicinal plants for cell viability

Before to investigate the NO activities, the effect of the 35 methanol extracts on the viability of RAW 264.7 cells were evaluated.

As shown in Table 3.6, *Mussaenda hoaensis* extract was showed the highest cell viability (132.73±0.14) on RAW 264.7 cells and *Catunaregam spinosa* (97.64±0.7), *Connarus semidecandrus* (96.50±0.85), *Justicia gendarussa* (94.85±2.49), *Fibraurea recisa* (93.11±9.64) and *Citrus* × *aurantium* (90.31±1.1) showed significantly high cell viability. On the other hand, *Eurycoma longifolia* (7.49±2.53), *Crinum asiaticum* (10.65±0.58), *Homonoia riparia* (11.55±2.04), *Lantana camara* (11.94±3.7) and *Elephantopus scaber* (19.99±1.46) were showed significantly low cell viability. These species are needed to study cytotoxic effect analysis in the near future for the development of anti-cancer drug.

3.3.1.2. NO inhibition activity

NO production was determined by Griess reagent assay. The NO production was measured in the medium of RAW 264.7 cells cultured with LPS in the presence of methanol extracts. Six of the 35 plants screened had anti-inflammatory activity above 80%.

Homonoia riparia extract was showed the highest NO inhibition activity (104.23 ± 0.25) and *Alstonia scholaris, Mussaenda hoaensis* and G*elsemium elegans* extracts were showed significantly high NO inhibition activity, corresponding to 99.56 ± 5.47, 98.07 ± 0.13, 96.57 ± 6.83, but reduced the cell viability in RAW 264.7 cells except *M. hoanesis* extract.

NO inhibition activity was more than 40%, and cell viability rate of more than 60% were *Fibraurea recisa, Blumea balsamifera, Mussaenda hoaensis, Embelia ribes* and *Catunaregam spinosa* medicinal plants. So, on the basis the current results, it can be suggested that *F. recisa, B. balsamifera, M. hoaensis, E. ribes* and *C. spinosa* medicinal plants can be used as a potent anti-inflammatory activity.

3.3.2 Screening of ethnomedicinal plants for anti-oxidant activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay is one of the most popular antioxidant assays used by almost 90 % of antioxidant studies (Moon & Shibamoto, 2009). The DPPH radical scavenging activity of methanol extracts derived from medicinal plants is shown in Table 3.8. *Homonoia riparia* extract was showed the highest antioxidant activity (75 ± 1.2) and *Vernicia fordii* (65 ± 0.8), *Psidium guajava* (46 ± 0.1) and *Macaranga denticulata* (41 ± 2.9) were showed high anti-oxidant activity.

3.3.3. Screening of ethnomedicinal plants for anticancer activity

3.3.3.1. Anticancer activity on lung cancer cell line

The results relating to cytotoxicity on human lung cancer cells (A549) are shown in Table 3.9. *Fibraurea recisa* extract was showed the highest anticancer activity (60.86) on A549 cells. In addition, stomach cancer activity experiment was showed high activity (35.83). *Cissus quadrangularis* (54.51), *Artocarpus heterophyllus* (52.9), *Elephantopus scaber* (48.1), *Spatholobus suberectus* (46.64) and *Eurycoma longifolia* (45.3) were showed significantly high anticancer activities on A549 cells.

3.3.3.2. Anticancer activity on stomach cancer cell line

The results relating to cytotoxicity on human stomach cancer cells (AGS) are shown in Table 3.8. *Lantana camara* extract was the showed the strongest cytotoxic activity (51.6, 43.2) on AGS cells and liver cancer cells (HepG2) and *Cissus quadrangularis* (41.34), *Choerospondias axillaris* (41.3), *Macaranga denticulata* (39.94), *Crinum asiaticum* (38.29), *Fibraurea recisa* (35.83) and *Scoparia dulcis* (35) were showed high anticancer activities on AGS cells.

3.3.3.3. Anticancer activity on liver cancer cell line

The results relating to cytotoxicity on human liver cancer cells (HepG2) are shown in Table 3.8. *Lantana camara* extract was showed

the highest cytotoxic activity (43.2) on HepG2 and *Gelsemium elegans* (39.7), *Crinum asiaticum* (37.02), *Elephantopus scaber* (35.8) and *Cissus quadrangularis* (27.66) were showed high anticancer activities on HepG2 cells.

3.3.3.4. Anticancer activity on breast cancer cell line

The results relating to cytotoxicity on human breast cancer cells (MCF7) are shown in Table 3.8. *Vernicia fordii* extract the showed the strongest cytotoxic activity (67.56) on MCF7 and *Crinum asiaticum* (54.31), *Elephantopus scaber* (51.8) and *Spatholobus suberectus* (42.37) showed significantly high anticancer activities on MCF7 cells.

Table 3.9. Results of anticancer activities on A549, AGS, HepG2 and MCF7 cell lines from selected 29 extracts

No.	Family	Scientific name	Anticancer activity			
			Lung cancer (A549)	Stomach cancer (AGS)	Liver cancer (HepG2)	Breast cancer (MCF7)
1	Acanthaceae	*Justicia gendarussa*	22.6	14.5	-	-
2	Amaranthaceae	*Crinum asiaticum*	39.35	38.29	37.02	54.31
3	Anacardiaceae	*Choerospondias axillaris*	19.7	41.3	17.9	33.9
4	Apocynaceae	*Alstonia scholaris*	15.6	13.9	13.8	22.1
5	Apocynaceae	*Dregea volubilis*	8.36	1.87	-	-
6	Apocynaceae	*Streptocaulon juventas*	5.3	16.1	15.5	23.7
7	Araliaceae	*Schefflera heptaphylla*	27.51	2.69	2.24	27.51
8	Compositae	*Blumea balsamifera*	11.52	22.81	-3.77	15.4
9	Compositae	*Chromolaena odorata*	5.03	14.39	18.65	4.78
10	Compositae	*Elephantopus scaber*	48.1	25	35.8	51.8
11	Euphorbiaceae	*Homonoia riparia*	30.5	30.1	18.4	15.1
12	Euphorbiaceae	*Jatropha curcas*	31.44	13.29	7.99	-1.97
13	Euphorbiaceae	*Macaranga denticulata*	14.53	39.94	2.35	12.86
14	Euphorbiaceae	*Vernicia fordii*	3.8	32.22	-	67.56
15	Gelsemiaceae	*Gelsemium elegans*	7.2	10.9	39.7	13.1
16	Lauraceae	*Phoebe tavoyana*	32.17	21.98	-9.55	20.9
17	Lauraceae	*Litsea cubeba*	26.76	19.27	-2.66	20.96

18	Icacinaceae	*Iodes cirrhosa*	7.9	18.68	-	-
19	Leguminosae	*Spatholobus suberectus*	46.64	8.35	19.9	42.37
20	Menispermaceae	*Fibraurea recisa*	60.86	35.83	13.62	0.38
21	Moraceae	*Artocarpus heterophyllus*	52.9	24.5	25.3	29.9
22	Myrtaceae	*Psidium guajava*	13.2	-	7.59	-
23	Plantaginaceae	*Scoparia dulcis*	30.4	35	9.6	18.7
24	Primulaceae	*Embelia ribes*	7.65	11.22	1.75	-11.82
25	Rubiaceae	*Catunaregam spinosa*	5	3.6	1.76	7.77
26	Rutaceae	*Citrus × aurantium*	7.1	16.4	-	-
27	Simaroubaceae	*Eurycoma longifolia*	45.3	23.37	1.8	30.19
28	Verbenaceae	*Lantana camara*	11.7	51.6	43.2	29.4
29	Vitaceae	Cissus quadrangularis	54.51	41.34	27.66	26.3

CONCLUSIONS AND FUTURE PROSPECTS

This is the first-ever study to document the traditional medicinal knowledge of the Van Kieu ethnic people from Bac Huong Hoa Nature Reserve, Vietnam. The study highlighted the richness of indigenous medicinal plant species and importance of associated traditional medicinal knowledge of the Van Kieu ethnic people. A total of 111 medicinal plants were reported by the Van Kieu people belonging to 46 families and 102 genera. The study reported medicinal properties of eight species for the first time. The study reported 41 species for treating different ailments than that of DVM. The study revealed that the Van Kieu people used some species in single prescription while few are used in polyprescriptions. They also used a mixture of some species to cure diseases. The Van Kieu ethnic people rely on herbal remedies for their basic health care, thus indigenous medicinal plant knowledge plays a vital role in solving local health care problems.

The study also was verification the antioxidant, anti-inflammatory and anticancer effects of ethnomedicinal plants in BHHNR. Especially, NO inhibition activity was more than 40%, and cell viability rate of more than 60% were *Fibraurea recisa*, *Blumea balsamifera*, *Mussaenda hoaensis*, *Embelia ribes* and *Catunaregam spinosa* medicinal plants and *Homonoia riparia* was the most anti-oxidant active among the selected plants.

Moreover, while studying the cytotoxic effects on human cancer cell lines (A549, AGS, HepG2, MCF7). it was evident that *Fibraurea recisa*, *Lantana camara*, *Gelsemium elegans* and *Vernicia fordii* had high cytotoxicity. Based upon the initial screening work reported here, further studies aimed at the identification of active components of four extracts and the elucidation of their mechanisms as cancer therapeutics are warranted.

In conclusion, the results obtained from our screening confirm the

therapeutic potency of the 9 plant species analysed and thus provide a rationale for their use in traditional medicine. Also suggest that ethnomedicinal plants from BHHNR in vietnam have antioxidant, anti-inflammatory and anticancer agents. The present results suggest that ethnomedicinal plants from BHHNR in vietnam have antioxidant, anti-inflammatory and anticancer agents. It can be suggested for future perspectives that several other methods should be applied to evaluate the same as it requires more investigation on the isolation and identification of antioxidant, anti-inflammatory and anticancer compounds and also in vivo studies for a better understanding of their mechanism of action.

The study attempted to document the traditional medicinal knowledge left in the community. Further, the data generated from this study will provide a basis for phytochemical and pharmaceutical studies and conservation of important medicinal plant including the endangered species in the study area.

LIST OF PUBLICATIONS

1. Do Van Hai, **Changyoung Lee**, Ha Minh Tam (2017), "Morphological Characters and Taxonomy of *Rhinacanthus* (Acanthaceae) in the Flora of Vietnam", *Proceedings of the 7th National Scientific Conference on Ecology and Biological Resources.* ISBN: 978- 604-913-615-3, pp. 140-144.

2. **Lee C.**, Kim S.Y., Eum S., Paik J.H., Bach T.T., Darshetkar A.M., Choudhary R.K., Hai D.V., Quang B.H., Choi S., Thanh N.T. (2019), "Ethnobotanical study on medicinal plants used by local Van Kieu ethnic people of Bac Huong Hoa nature reserve", *Vietnam. J. Ethnopharmacol*. 231, pp. 283-294. (SCI)

3. **Lee C**, Kim Y.I., Kim S.Y., Bach T.T., Eum S., Thanh N.T., Choi S. (2019), "The complete chloroplast genome sequence of a vulnerable legume species *Dalbergia tonkinensis* Prain in Vietnam", *Conservation Genetics Resources* (SCIE)

4. **Changyoung Lee**, Tran The Bach, Do Van Hai, Bui Hong Quang, Bui Thu Ha, Nguyen Trung Thanh (2019), "Assesment of Bioactivity of Some Plant Species in Bac Huong Hoa Nature Reserve and its near Places of Quang Tri Province", *VNU J. Science, Vietnam*, Vol. 35(1), pp, 1-8.