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PhD Project

Studi etnobotanici ed etnofarmacologici sulle piante medicinali usate nelle aree rurali del Distretto di Kavrepalanchok (Nepal Centrale)

Ethnobotanical and ethnopharmacological studies of medicinal plants used in rural areas of Kavrepalanchok District (Central Nepal).

PhD candidate: Gabriele Ambu

Tutor: Prof.ssa Laura Cornara

Coordinator of the PhD course: Prof. Marco Firpo

Coordinator of the PhD school: Prof. Mauro Mariotti

ABBREVIATIONS AND ACRONYMS

AChE	Acetylcholinesterase enzyme
AHC	Agglomerative hierarchical clustering analysis
CBS	Central Bureau of Statistics
CITES	Convention on International Trade in Endangered Species
CNS	Central Nervous System
DISTAV	Department of Earth, Environment and Life Sciences (Genova)
EHTPA	European Herbal and Traditional Medicine Practitioners Association
ENT	Ear, nose and throat
EO	Essential oil
Fic	Informant Consensus Factor
FL	Fidelity Level
GC	Gas Chromatography
GC-MS	Gas Chromatography- Mass Spectrometry
IECPS	1st International Electronic Conference on Plant Science, 01-15/12/2020
MAPs	Medicinal and aromatic plants
MBR	Mean Burst Rate
MFR	Mean Firing Rate
MEA	Microelectrode arrays technique
Nj	Nardostachys jatamansi
PCR	Polymerase Chain Reaction
PNS	Periphery Nervous System
RECAST	Research Center for Applied Science and Technology
RFCs	Relative frequency of citation
SEM	Scanning electron microscopy
TEK	Traditional Ecological Knowledge
TUCH	Tribhuvan University Central Herbarium

UN	United Nations
UNIME	University of Messina (Department of Chemical, Biological, Pharmaceutical and Environmental Sciences).
UNIMI	University of Milano (Department of Biotechnology and Bioscience)
Vj	Valeriana jatamansi
Vo	Valeriana officinalis
WBD	The World Bank Data
WHO	World Health Organization

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Abstract

The current economic and social conditions in many rural areas of the world are threatening the precious heritage of ethnobotanical knowledge and traditional farming practices. This can cause loss of precious cultural heritage and reduction in plant biodiversity, as ancient crops tend to disappear.

The main aim of this thesis is to document traditional uses of plants by different ethnic groups (Tibeto-Burman and Indo-Aryan) living in certain rural areas of the Kavrepalanchok District in Central Nepal. In the study area, due to distance from urban centres and difficulty in accessing the government healthcare system, people still rely heavily on the use of local plants for various purposes, above all for primary healthcare.

Through interviews with 32 informants, most of whom were key informants, we explored uses of 116 plant species, of which 101 were plants with medicinal value employed in the treatment of human and veterinary diseases. Some unusual uses of medicinal plants and original recipes were also reported.

The data document the richness of the local flora and traditional knowledge of medicinal plant species used by ethnic communities in these rural areas.

Therefore, future projects will have to involve local people in the improvement and conservation of the biological and cultural heritage. There is also a need for an ecological strategy for integrated management of land, water and living resources.

Another aim of the research presented in this thesis is to better characterise some plants found to be of particular interest among those surveyed in the study area. With this in mind, we have focused our attention on those plants used by informants in the treatment of nervous system disorders, such as two species belonging to Caprifoliaceae (formerly Valerianaceae): *Valeriana jatamansi* Jones ex Rob. and *Nardostachys jatamansi* (D. Don) DC. These plants are widely used in traditional medicine for their sedative and anxiolytic properties in Nepal and in many other Asian countries. The pharmacognostic and phytochemical profile and the biological effects of essential oils (EOs) of these species were compared with those of *Valeriana officinalis* L., a species whose phytotherapeutic use is widespread in Western medicine.

The multidisciplinary approach used represents a way to avoid adulteration of herbal drugs and allows evaluation of the effectiveness of EOs that could be used for a wide range of therapeutic applications.

Overall, the results of this research could be useful for enhancing knowledge of the potential of still little-known medicinal plants for the possible formulation of new pharmaceutical products, eventually contributing to the economic development of local communities.

Introduction

1. Background

The term “ethnobotany” appears for the first time in an article by the botanist John W. Harshberger published in the Botanical Gazette (1896) and entitled “The purposes of Ethnobotany”, where the author defined ethnobotany as “the study of plants used by primitive and aboriginal people”. In a wider sense, this discipline can be considered as the set of studies concerning the relationship between people, plants, and the environment. Ethnobotany is a discipline that has developed significantly over the years, and involves a wide range of other branches of studies such as plant taxonomy, nutrition, pharmacognosy, phytochemistry, plant ecology, and conservation biology. With the progress of anthropology starting from the end of the 19th century, ethnobotany expanded its field of interest to include other aspects, such as the role of plants in folklore, literature, ceremonies and world view. Moreover, renewed interest was also found in the use of plants across societal subgroups based on age, gender, social status, and occupation.

Then, the environmental movement in the late 1960s and early 1970s caused ethnobotany to recognise the importance of the Traditional Ecological Knowledge (TEK) of indigenous and local populations for global strategies of sustainability and biodiversity conservation. In this period (1979) a book was published by an author who is considered the father of modern ethnobotany, *Plants of the Gods: Origins of Hallucinogenic Use* by Richard Evans Schultes (1915-2001).

Recently, ethnobotanists have begun to emphasise the use of plants from traditional systems of medicine. This has led to the development of phytochemical studies (ethnomedicine and ethnopharmacology) to identify their bioactive compounds and related therapeutic properties (Cotton, 1996; Martin, 1995; Nolan & Turner, 2011).

Traditional systems of medicine provide important healthcare all over the world, especially in developing countries (WHO, 2019). The most interesting ethnobotanical data are generally collected in ethnic communities living in rural areas of remote regions, where TEK often remains under-documented (Adhikari et al., 2019).

In Nepal, where 80% of the population live in rural areas (United Nations, 2019; World Bank Data, 2019) and it is difficult to access government healthcare facilities, people depend highly on the traditional use of medicinal plants for their primary healthcare. It is estimated that about 2,000 species of flowering plants are commonly used in traditional healing practices (Gaire et al., 2011).

In Nepal, the use of plants for medicinal purposes is very ancient, and traditional healing practices are still widespread today. The wide diversity of plants used and the wide range of

preparations available are closely linked to many cultural factors. Indeed, there are more than one hundred ethnic groups, each with its own culture, language, religious rites, and traditional practices in the use of medicinal plants (Central Bureau of Statistics (CBS), 2012; Niroula et al., 2015).

This important knowledge may be written and codified in medico-religious manuscripts (as in Ayurvedic, Tibetan, and Unani medicine systems) or passed down orally, mainly within families or small groups of healers (as in folk and shamanistic medicine) (Aryal et al., 2016).

Ayurveda is an ancient traditional medical system that originated between 1500 and 900 BC. It is widespread especially in urban centres, with its hospitals, dispensaries, and medicine manufacturing units. Tibetan medicine is influenced by Buddhist philosophy and is commonly practised in the mountainous regions by "amchis", Tibetan doctors. The Unani system is based on principles developed by the Greek physician Galen (2nd to 3rd century AD). This system of medicine has been enriched by the contribution of Arab and Persian doctors and philosophers. Unani practice is recognised by the government of Nepal. Folk and shamanistic systems of medicine are present in rural areas in particular. Folk medicine refers to the medical knowledge maintained and passed on by elderly people mainly, and has developed through a process of countless trial and error. Shamanic medicine is a spiritual healing system in which the shaman ("dhami-jhankri") acts as a mediator between the material and spiritual world to cure a person's illness (Gewali, 2008; Paudel, 2006).

The study of traditional knowledge concerning the use of medicinal plants by ethnic communities in Nepal began to develop in the 1960s. According to Rajbhandary et al. (2015), from 1968 to 2014 there has been 822 publications on Nepalese ethnobotany, of which about 360 are focused on medicinal plants. However, despite growing scientific interest in traditional medical practices, and although people from ethnic communities prefer traditional healers to modern hospitals or healthcare services, during the last fifty years, Western allopathic medicine has been slowly replacing traditional healing practices in rural areas (Baniya, 2014).

2. **Objectives**

This PhD project consisted of two parts.

In the first part, an ethnobotanical survey was carried out among people from ethnic groups in the rural areas of the Kavrepalanchok District (Central Nepal), with a particular focus on medicinal plants selected and used by local healers and shamans who are considered the keepers of TEK. Previous ethnobotanical studies were conducted in other villages of the same district and in different districts of Central Nepal (Table 1).

Table 1. Number of plant species documented in previous studies conducted in Central Nepal.

References	Species	Genera	Families
Kavrepalanchok District			
Bhattarai (1990)	36	36	27
Manandhar (1991)	95	88	47
Malla & Chhetri (2009)	68	59	37
Shah & Lamichhane (2017)	55	51	38
Other districts of Central Nepal			
Shrestha (1988)	100	89	58
Bhattarai (1989)	57	47	25
Shrestha & Dhillion (2003)	58	55	40
Tamang (2003)	44	41	27
Kunwar et al. (2006)	108	96	61
Joshi & Joshi (2008)	48*	35	29
Bhattarai et al. (2009)	94	86	49
Joshi et al. (2011)	87	79	54
Thapa (2012)	75	72	46
Luitel et al. (2014)	161	144	86
Tamang & Sedai (2016)	80	74	44

* Authors considered only mushrooms and ferns.

During the PhD research, data about 116 plant species were collected via interviews with local people. Most of the plants cited were also sampled for an ethnobotanical herbarium, then kept at Tribhuvan University. Subsequently, all the data were organised in a database and processed through appropriate internationally recognised ethnobotanical indices. In this first phase of the research, collaboration with the Research Centre for Applied Science and Technology (RECAST) at Tribhuvan University (Prof. Ram P. Chaudhary) was crucial.

In the second part of this PhD, the study of some plants known in the treatment of neurological disorders in the previously investigated area was deepened. My interest was focused on two Himalayan Caprifoliaceae species, *Valeriana jatamansi* Jones ex Rob. and *Nardostachys jatamansi* (D. Don) DC.

Both species are used in traditional medicine for their sedative and anxiolytic properties in Nepal and in other Asian countries. Roots and rhizomes of *V. jatamansi* are used in the treatment of mental disorders and epilepsy (Rather et al., 2012). In Ayurvedic medicine, *N. jatamansi* is used for its carminative, sedative, antispasmodic and tranquillising properties (Jha et al., 2012).

The pharmacognostic and phytochemical characteristics, and the biological effect of the essential oils of these species, were compared with those of the Eurasiatic species *Valeriana*

officinalis L. This plant has been used since ancient times in the treatment of various nervous disorders, especially in Western medicine (Nandhini et al., 2018).

The EOs of the species under consideration have been reported to contain several monoterpenoids and sesquiterpenoids, to which their pharmacological effects at the CNS level seems mainly attributable (Bhatt et al., 2012; Komori et al., 2006; Saroya & Singh, 2018). Indeed, some of these compounds have been shown to have a direct effect on the amygdala region or to inhibit the enzyme-induced breakdown of GABA in the brain, resulting in sedation (Houghton, 1999). However, the mechanisms that underlie the neuroactive effects of Valerian EOs are still poorly investigated. Considering this, and keeping in mind growing interest in these species on the botanicals market, this study aimed to carry out a multi-level comparison of the three species in terms of roots/rhizomes micromorphology, DNA barcoding and EOs phytochemical characterisation. In addition, we assessed the biological effects of these EOs on the nervous system by testing the acetylcholinesterase (AChE) inhibitory activity and by using the microelectrode arrays technique (MEA).

Plant material was obtained thanks to collaboration with the Nepal Herbs and Herbal Products Association (Kathmandu, Nepal, Dr. Ghimire Govinda), the European Herbal and Traditional Medicine Practitioners Association (EHTPA, Norwich, UK, Dr. Marco Valussi), and Biosline, Padova.

Micromorphological analyses were performed at the Department of Earth, Environment and Life Sciences (DISTAV) laboratories (University of Genova) using both optical and scanning electron microscopy techniques, while further characterisations were carried out in collaboration with other research teams: namely the

Department of Biotechnologies and Biosciences at the University of Milano Bicocca (UNIMI); the Department of Chemical, Biological, Pharmaceutical and Environmental Sciences at the University of Messina (UNIME); and ETT S.p.A. in Genova.

Plate 1. Together with the Prof. Dr. Ram P. Chaudhary (right side) at RECAST , Tribhuvan University.

Part I

Ethnobotanical survey on traditional uses of plants by people from ethnic communities living in rural areas of the Kavrepalanchok District in Central Nepal

Chapter 1: Materials and Methods

1.1.Study Area

Nepal is located between the latitudes of 26°22'–30°27' and longitudes of 80°04 '–88°12' and is bordered to the north by the Tibetan Autonomous Region of China and to the south, east, and west by India. Despite its small area of 147,181 km^2, due to its large altitude and climatic gradient, Nepal has a rich biodiversity of flora and fauna, as well as significant ethnic and cultural variety (Ghimire 2014; Manandhar 2002) (Figure 1).

Figure 1. Map of Nepal (Source: Map No. 4304 UNITED NATIONS January 2007).

The current Annotated Checklist of Flowering Plants of Nepal (2000) enumerates about 6,000 flowering species, but botanical experts estimate that there are more than 7,000 species, since many remote regions are still poorly explored (Tiwari et al., 2019). From a phytogeographical point of view, this high diversity is linked to the transitional position that Nepal occupies between different floristic zones: the western Himalaya, the eastern Himalaya, the Tibetan plateau to the north, and the Gangetic Plains of India to the south (Tiwari et al., 2019; Welk, 2015).

This PhD research concerned the use of plants by different ethnic groups living in certain villages in the mid-hilly area of the Kavrepalanchok District, in the Bagmati Pradesh Province of Central

Nepal. This district is located between 85°24'–85°49' E and 27°22'–27°85' N, with altitudes ranging from 275 (Dolalghat/Sunkoshi River) to 3018 m ASL (Bethanchowk Hill). The total area is about 1396 km^2 and the average temperature ranges from 10 °C to 31 °C (Manandhar, 1991). It is an area corresponding to a subtropical zone, with a monsoonal climate and average annual rainfall of 1,000–2,000 mm.

The vegetation is characterised by pine and broad-leaved evergreen forests (Stainton, 1972) of *Pinus roxburghii* Sarg., *Schima wallichii* Choisy, *Castanopsis indica* (Roxb. ex Lindl.) A.DC., *Toona hexandra* (Wall.) M.Roem., *Alnus nepalensis* D.Don at the lower belt, while broad-leaved oak forests of *Quercus* spp. are found at the upper belt (Bajracharya, 1996) (Figure 2).

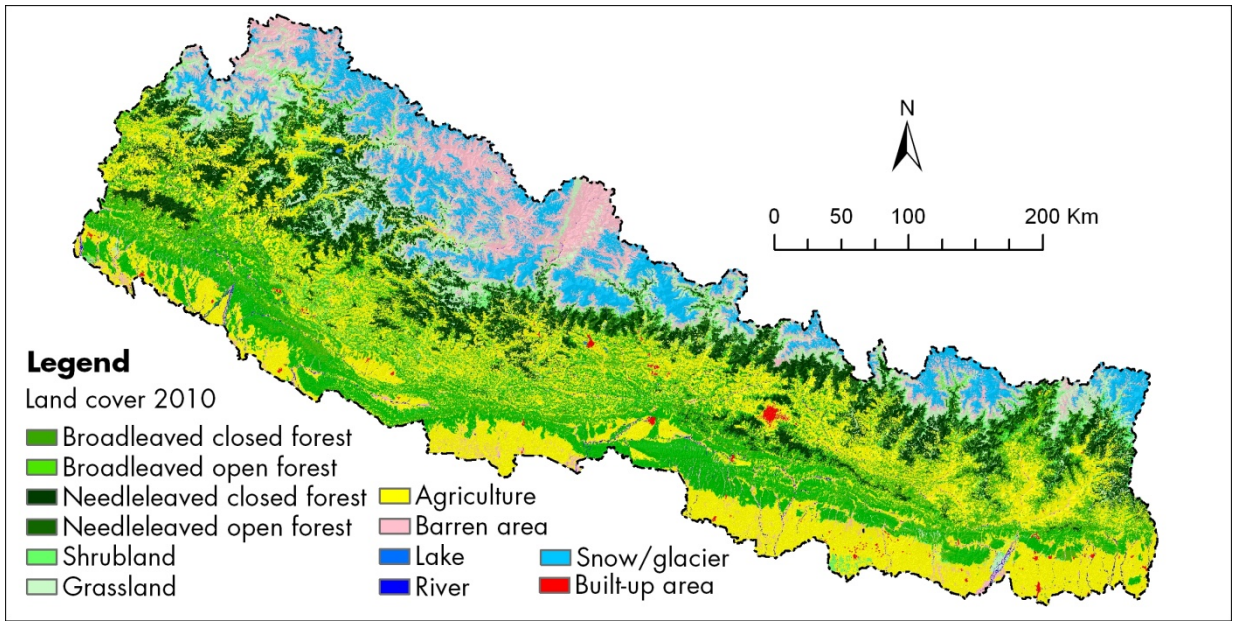

Figure 2. Land cover map of Nepal (Uddin et al., 2015).

Within this district, the area of investigation for this ethnobotanical research was specified in accordance with certain criteria. Eight rural villages were chosen, in order to have a sufficiently homogeneous study area in terms of culture and land use. Furthermore, the choice of area was commensurate with data collection methods, time available for the research, logistical difficulties, number of researchers involved, economic resources, etc. Before proceeding with the collection of data on traditional uses of plants, knowledge of the environment and culture of the place was deepened via bibliographic research, preliminary travels, and participation in the daily life of local people (Camarda & Guarrera, 2005; Cotton, 1996).

Plate2. Landascapes variety from the survey area. (a) Sun koshi river; (b) Kathmandu Valley from Dhulikhel.

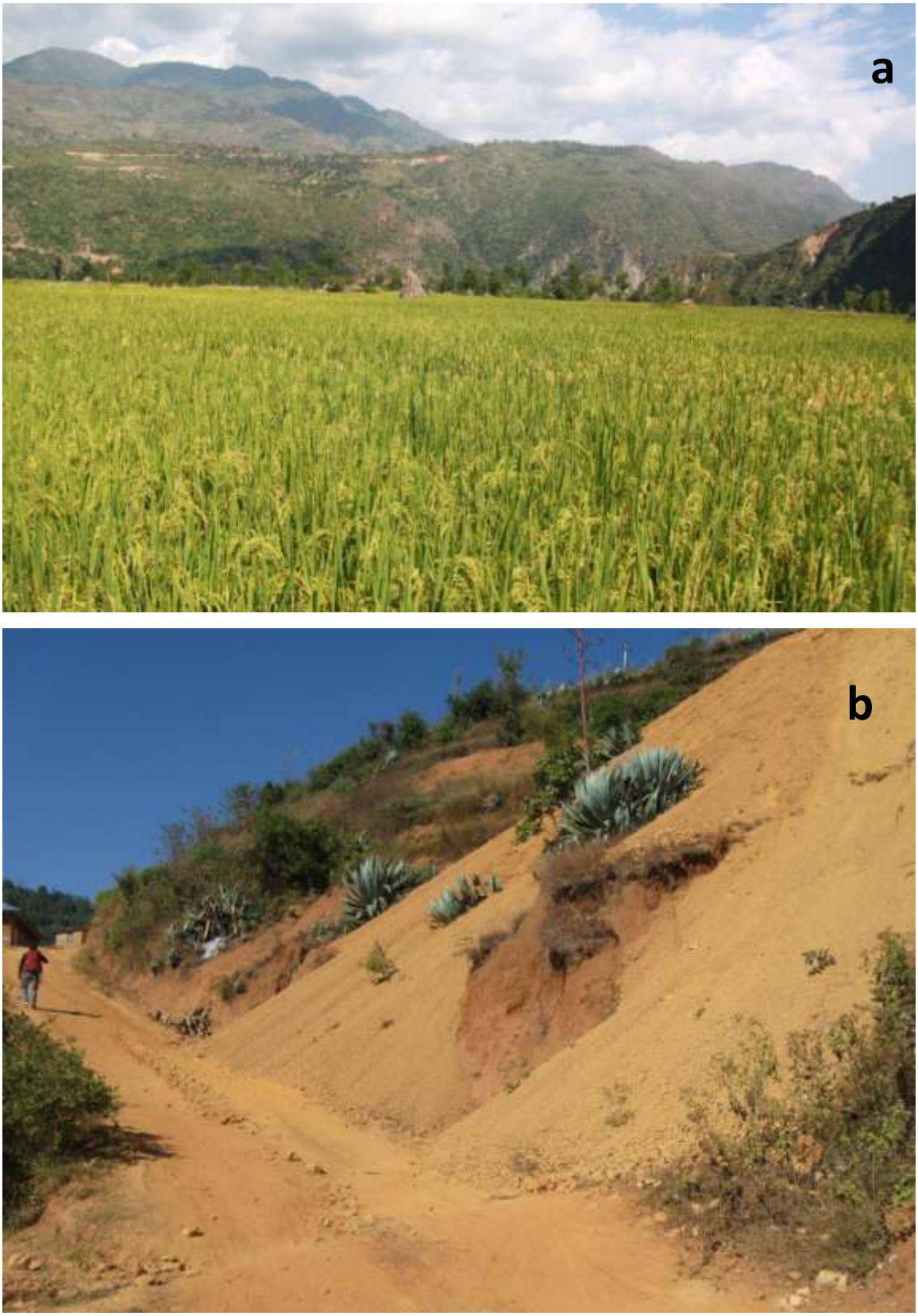

Plate 3. Vegetation diversity in Kavrepalanchok district: rice field along the river (a) and xerofitic vegetation at higher altitudes (b).

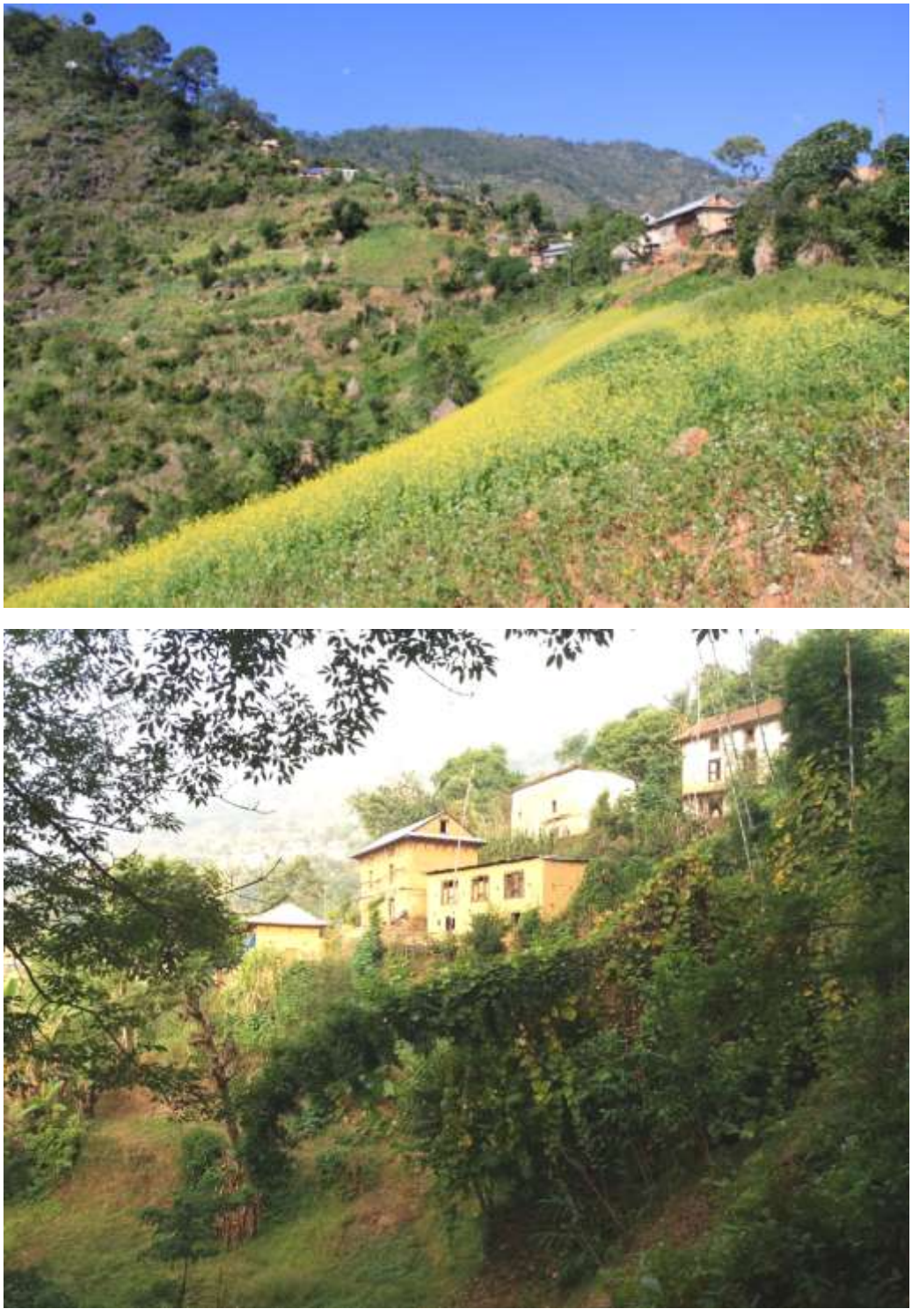

Plate 4. Villages of the survey area in Kavrepalanchok District.

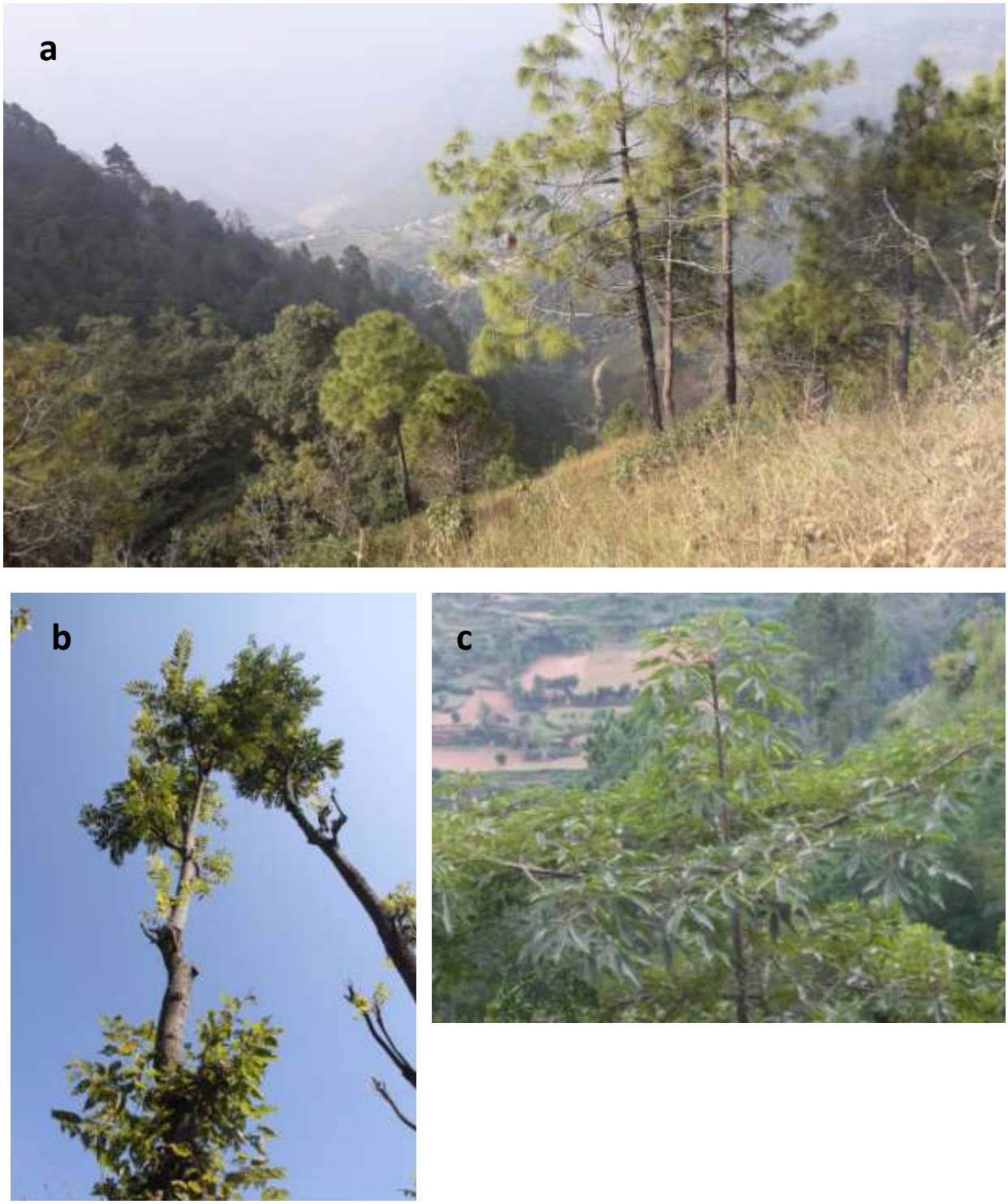

Plate 5. *Pinus* spp. (a) and deciduous trees forests of *Toona hexandra* (b) and *Bombax ceiba* (c).

1.1.1. **Ethnic groups**

At the time of the 2011 Nepal census, Kavrepalanchok District had a population of 381,937: 50.9% speaking Nepali, 33.5% Tamang, 11.1% Newari, 1.6% Danuwar and 1.4% Magar as their first language (CBS, 2012). Rural villages chosen for the survey are about 100 km from Kathmandu, with the Tamang community representing the main ethnic group, while other groups represent minorities. In accordance with this ethnic composition, our informants mainly belonged to the Tamang ethnic group, and to a lesser extent to other minority communities living in the study area, such as the Newar and Brahmin communities.

The study area is not too far from city centres, but people living here are very much deprived of basic infrastructures and facilities. Many people are illiterate and therefore the majority of the population are involved in agriculture, mostly subsistence farming. Some people work in the tourism industry as porters, guides and drivers, among other occupations. Since there is a lack of educational facilities for children, and their parents often consider education as a trivial factor in life, children are not encouraged to get an education.

Each ethnic group still holds important folklore about uses of plants for various purposes. It is estimated that in Nepal, more than 2,300 plant species are used by local people as traditional medicine to cure various diseases, and many of them are regarded by the scientific community as potential sources for new therapeutic drugs (Kandel et al., 2020).

Tamang

The Tamang is one of the major Tibeto-Burmese speaking communities in Nepal and, according to a well-known Nepalese myth, are descended from the god Siva (Peters, 2007). Their name in Tibetan means horse traders, and they probably came from Tibet in the 6th century and later settled in the fertile hilly areas adjacent to the Kathmandu Valley. It is believed that their first place of settlement was the southern flank of Ganesh Himal (Toffin & Wiart, 1985). Tamang people have rich traditional knowledge about plants and animals. Nevertheless, only a few ethnobotanical studies have been carried out concerning this culture (Luitel et al., 2014; Malla & Chhetri, 2009; Shrestha, 1988; Shrestha & Dhillion, 2003).

The Tamang have an animistic view of the world in which the world is populated by numerous spirits living in nature responsible for many events in life. Evil spirits, angry ghosts (“pichas”), and witches (“bokshi”) are considered to be the cause of balance alterations in the human organisms that cause diseases. As in other studies conducted in neighbouring areas (Ghimire, 2014), it is common also in this study area to find certain plants above door thresholds

doors — plants such as “tatelo” (*Oroxylum indicum* (L.) Kurz.) and “kurilo” (*Asparagus racemosus* Willd.) — to protect from the evil eye. Other plants are used as propitiatory symbols of good luck.

From this animistic world view, shamans (“jhakris-bombo”) play a very important role in Tamang communities. They act as mediators between the spiritual and the material world and have developed: specific techniques to identify evil spirits, rituals to expel them such as “mantra” (secret whisper) and amulets, and herbal preparations for the treatment of humans and animals (Khatry, 2011; Peters, 2007). During this research, we had the opportunity to actively participate in the healing rites of some shamans, and we noticed that many of the plants linked to rituals are also used in traditional medicine to treat various human diseases.

Newar

Newar people are considered to be the indigenous inhabitants of the Kathmandu Valley. They are of Tibeto-Burman origin, and are the ethnic group to which Sakyamuni Buddha, the founder of Buddhism, belonged. It is believed that the Newar settled in the Kathmandu Valley, migrating from the southern Terai region, shortly after the birth of the Buddha in Lumbini, on the border with India. The Newar are mainly involved in agriculture and plant trade. Furthermore, they have a rich artistic and cultural tradition, with rituals linked to the life cycle (Dietrich, 1998; Manandhar, 2002) and to many plants with a religious value.

For example, there is a ritual called “Ihi”, a symbolic marriage of Newar girls with the god Shiva symbolised by the *Aegle marmelos* (L.) Corrêa (“bel”) fruit (Niroula & Singh, 2015; Shakya, 2006). As another example, during the festival for the end of the rice planting season celebrated in the Kathmandu Valley (“Gathemanga”), Newar people place pots of cooked rice along with *Leucas cephalotes* (Roth) Spreng. (“topdoi mra”) flowers at the crossroads as offerings to the evil spirit (Rajbhandary & Winkler, 2017).

Newar people also have an important tradition of tantric healing. Tantric healers (“guvaju-deovaju”) are considered people who are able to connect with the supernatural realm and intercede for the healing of humans (Malla, 2015; Phuyal, 2006; Subedi, 2012).

Brahmin

Brahmin (“Bahuns” in the Nepali Language) represent one of the Hindu Nepalese castes rather than a distinguished ethnic group. They are of Indo-Aryan origin and live in the central area of Nepal, occupying fertile agricultural land at the foot of the Himalayan mountain range. All Brahmin people are Hindus and celebrate festivals, and perform rituals and ceremonies, where Brahmin men often play the role of priests, and plants are crucial for the celebrations. For example,

a typical plant used for the spiritual purification of environments is “tulsi” (*Ocimum tenuiflorum* L.). Most of the plants that are related to rituals, ceremonies and festivals are important from a medicinal point of view (Fisher, 1992; Sapkota, 2013).

1.1.2. **Traditional medicine in Nepal**

The World Health Organization (WHO) defines Traditional Medicine (TM) as follows: “the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement, or treatment of physical and mental illness”.

Bechan et al. (2011) showed that there is a very significant variety of traditional medicine systems in Nepal. The choice people make depends on various factors such as the type of illness, the availability of the remedy, personal beliefs etc. Generally, the Nepalese traditional system of medicine can be classified into medical systems of different schools (Ayurvedic, Tibetan and Unani), folk medicine and shamanistic systems.

The term Ayurveda means “science of life” and is an ancient system of medicine based on the principles of Hinduism, whose origin goes back to the Vedic period. The most important texts of the Ayurveda, Charaka Samhita and Susruta Samhita, were written between 1500 BC and 300 AD, and describe therapeutic remedies for human diseases, including medicinal plants, massage, ointments, enemas, surgery etc. (Gewali, 2008; Kunwar & LeBoa, 2017; Shankar & Paudel, 2006). Today, Ayurveda is recognised by WHO, and in Nepal there are many Ayurvedic dispensaries and health centres.

The Tibetan medical system is also known as Sowa Rigpa (knowledge of healing) and combines elements from the Hindu Ayurvedic system with Buddhist and other medical systems. Tibetan doctors are called “amchis” and their medical training is either passed down or obtained in official institutions. Tibetan medicine treatments include diet, herbal remedies, surgery, massage, moxa, and various types of acupuncture on select points of the body. Tibetan medicine has spread in Nepal for a long time, especially after the Dalai Lama's exile in 1959, when many Tibetan refugees arrived in Nepal and helped promote this traditional healing system (Gewali, 2008; Shankar & Paudel, 2006).

Unani is a system of medicine of Greek origin and systematised by Hakin Ibn Sina (Avicenna) in 980 AD in Persia. Later, in the nineteenth century, Ajmal Khan helped spread Unani medicine in India. Currently, this system of medicine is widely practised in Persia, Pakistan, India, South Africa, England, as well as other countries in the world. In Nepal, despite being recognised by the government, it is not very widespread. Unani healing methods include regimental therapies

(such as diaphoresis, diuresis, Turkish baths, massage, cauterisation, and purging), diet therapy (foods to be taken and foods to be avoided), pharmacotherapy (mainly herbal drugs and some animal and mineral products) and surgery (Gewali, 2008; Lloyd, 2009).

Folk medicine refers to the rich knowledge of ethnic groups about treating many diseases common to man and animals. This system, based on experience, and passed down orally from one generation to the next, includes use of medicinal plants, minerals, and animal products (Aryal, 2016; Bechan et al., 2011; Gewali, 2008).

Shamanism, also known as a faith healing system, represents the most important feature of traditional medicine in rural areas of Nepal and is particularly effective in cases of mental distress and emotional tension. In shamanistic medicine, the religious aspect is prevalent and concerns the belief that a person's illness is caused by spirit possession. Therefore, the shaman combines herbal medicine and faith — prayers (mantras), worshipping specific gods, sacrificing animals, astrology, amulets ("buti"), etc. But the shaman knows hundreds of medicinal plants too and provides herbal remedies to those who resort to his services. He knows not only which plants to use to cure diseases, but also the harvest time, the useful parts, the quantity of individual ingredients required for medicinal recipes etc. Shamans are called by the general term "dhami-jhakri", but in each ethnic group they are known by specific names, such as "guvaju/kaviraj" in Newari, or "bombo" in Tamang. These traditional healers may learn healing techniques from sources including elderly or neighbours, but often they claimed that they obtained their knowledge from supernatural forces via meditation and dreams, or that their knowledge was taught by deity ("ban-jhakri") in the forest. A wide range of techniques exist within different ethnic communities, as well as among different healers within the same community. Despite this diversity, it is not uncommon that shamans of one ethnic group perform healing rituals for another different group (Aryal et al., 2016; Gewali, 2008; Peters, 2007; Rajbhandary & Winkler, 2015).

Plate 6.

Buildings in Kavrepalanchok district damaged by earthquake of April 2015.

Plate 7. In the rural areas, besides agriculture, buffaloes, goats and chickens are the main sources of livelihood.

Plate 8. Rural houses.

Plate 9. Tamang people: a man preparing simple food in a rural house (a) and a woman with her child (b).

Plate 10. Newar people engaged in wood crafts.

Plate 11. Tamang girl collecting medicinal plants.

1.2.Field survey and data collection

This study was carried out in the months of October and November, after the monsoon season, in 2017 and 2018.

During two previous study trips (in 2015 and 2016), I spent a period in the rural community to become familiar with different aspects of the community — family organisation, daily habits, ways of living and relating to one other, work, relationship with the environment and health, as well as festivals and ceremonies linked to religious beliefs and cycles of nature (Ambu et al., 2017; Cotton, 1996). In addition, in this period we contacted RECAST of Tribhuvan University and signed a "letter of intent" with Prof. Ram P. Chaudhary, in order to have his supervision in the field of activity, particularly for taxonomic identification of plants.

1.2.1. Interviews

Ethnobotanical and ethnomedicinal data on plant uses were gathered by talking to people, and observing and participating in their daily activities (Alexiades, 1996; Martin, 1995). We interviewed 32 informants from the different villages of the study area. For the selection of the typology and number of informants, we followed the "purposive sampling" approach of Tongco (2007), a non-random method where informants are selected by virtue of knowledge and experience. In our case, such key informants were persons recognised by the community as keepers of traditional knowledge about medicinal plants. This approach allowed us to collect a high amount of reliable data with a low number of interviews. Key informants (24) were traditional healers selected by the following criteria: experience (local healers and shamans), age (knowledgeable elder villagers and occupation (farmers and plant traders). Interviews were conducted in the Tamang and Nepali languages with the help of a bilingual local guide. The data were collected in compliance with the rules of the World Intellectual Property Organization (WIPO) related to traditional cultural expressions (WIPO, 2004), and the ethical guidelines of the International Society of Ethnobiology (ISE) related to investigation and exploitation of biological diversity (ISE, 2021). The purposes and modalities of the research were carefully communicated to the informants and prior informed consent (PIC) was obtained (Singh et al., 2013). We explained to each informant that the information would be collected anonymously and that it would be used for non-profit research. We obtained permission to take notes and record interviews and daily activities with a camera.

Different interviews and inquiry methods were followed, in accordance with Alexiades (1996) and Martin (1995):

(a) informants were asked to freely list all plants of a certain ethnobotanical interest, with special attention to plants used for medicinal purposes (free listing);

(b) healers and shamans were accompanied on their trips in search of medicinal plants and followed during the healing ceremonies and preparation of herbal remedies (walk-in-the-woods and participant observation). Some difficulties were experienced in interviews with shamans because of their belief that revealing the medicinal properties of a plant deprives it of therapeutic efficacy;
(c) samples of plants were shown to the informants, asking them to identify species of ethnobotanical interest (specimen display);
(d) small groups of people were interviewed about their ethnobotanical knowledge using the specimen display method (group interviews).

All information was collected via semi-structured questionnaires. However, the specific questions were not asked in a predetermined order, but rather in the context of a free interview. This approach was chosen to avoid making informants uncomfortable, to avoid making them feel that they were the "object" of study, and to avoid asking culturally inappropriate questions (Edwards et al., 2005).

In this way, the informant was left free to remember and tell, bringing out elements that would otherwise have remained unexpressed within a more rigid interview scheme. Moreover, thanks to the climate of mutual trust, even the shamans who in some cases had been more reluctant to communicate their "secret" knowledge did share some data.

The questionnaires included questions about the informants (gender, age, ethnic group, occupation, educational level, birthplace, place of residence, location of interview, etc.), and the plants (local name, growth form, habitat, parts used, uses, preparation and routes of administration).

Plate 12. Some informants of this PhD reasearch.

Plate 13. Female shaman.

Plate 14. Shaman engaged in healing rituals with his tools.

Plate 15. Moments of ethnobotanical survey. Interviews with indigenous people (a) and discussion group (b).

1.2.2. Collection and identification of plant species

Plant specimens were collected with the assistance of local people, taking into account the presence of protected and endangered species, and using the checklist of CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) species in Nepal (Joshi et al., 2017). Standard techniques for collecting and preparing herbarium specimens were employed: a field press and some newspaper sheets to store the collected samples; a pair of scissors and a shovel for the extraction of plants and their underground parts; and paper bags to store seeds, fruits, or other parts of plants useful for species identification. Each plant collected was photographed (using a Canon PowerShot A590) and a label was prepared, indicating the local name, date, and place of collection (latitude and longitude), as well as habitat features and a progressive collection number.

The plants were identified in the field with the help of books on the native plants of Nepal (Joshi & Joshi, 2006; Malla et al., 1986; Manandhar, 2002; Polunin & Stainton, 1999) and then brought into the laboratory of RECAST at Tribhuvan University for carrying out correct identification and comparison with relevant specimens deposited in the Tribhuvan University Central Herbarium (TUCH). The plant nomenclature follows that of Plants of the World[1].

1.2.3. Herbarium Preparation

The set of plant specimens collected was prepared following standard techniques provided by the British Columbia Ministry of Forests (1996) and deposited in TUCH, with voucher numbers, following the guidelines of University of Florida Herbarium[2].

1.3. Quantitative analysis

After completing the field work, all ethnobotanical information related to informants, plants (scientific and vernacular name, family, local name, habitat, voucher specimen number, parts used) and traditional and ethnomedicinal uses was standardised and organised in a database (Microsoft Office Excel) for further analysis. The data were then processed into ethnobotanical indices of cultural relevance —Relative Cultural Importance (RCI) — aimed at quantifying the use of species or botanical families to establish their importance as a resource for local populations.: informant consensus factor (Fic), fidelity level (FL), relative frequency of citation (RFCs), as follows:

[1] Plants of the World Online. Royal Botanic Gardens, Kew. Available online: http://plantsoftheworldonline.org/ (accessed on 30 April 2020).
[2] https://www.floridamuseum.ufl.edu/herbarium/voucher.htm.

1.3.1. Informant consensus factor (Fic)

Trotter & Logan (1986) developed a method based on the concept of "informant consensus" to identify potentially effective medicinal plants. Informant consensus factor was calculated in order to find out the level of homogeneity in the information provided by informants. This index is calculated for each ailment category with the following formula (Collins et al., 2006; Trotter & Logan, 1986).

$$Fic = (Nur - Nt)/(Nur - 1)$$

where Nur is the number of use reports in a particular illness subcategory, and Nt is the number of taxa or species used in the same subcategory. A high Fic value indicates the level of agreement among the informants on the use of taxa within a medicinal subcategory.

1.3.2. Fidelity level (FL)

To determine the plant species most frequently used for the treatment of a particular disease category by the local population of the study area, the fidelity level (FL) was calculated. FL is the percentage of informants claiming use of a certain plant for the same major purpose, and is calculated according to the following formula (Alexiades, 1996; Andrade-Cetto & Heinrich, 2011):

$$FL = Np/N \times 100$$

where Np is the number of informants that claim to use a plant species to treat a particular disease, and N is the number of informants that use the same plant as medicine to treat any disease.

1.3.3. Relative frequency of citation (RFCs)

This index is used to determine the local importance of each species in the study area. The formula used, according to Tardìo & Pardo-de-Santayana (2008) is:

$$RFCs = FCs/N$$

where FCs is the number of informants that cite the use of a plant species, and N is the total number of informants.

Chapter 2: Results

A total of 32 informants, spread across the villages of the study area, of different genders (26 men and 6 women), ethnicity (both Tibeto-Burman and Indo-Aryan), age (23–81) and education level, were interviewed. Among them, 62% had a certain level of education (primary or secondary school) and provided 78% of the ethnobotanical information, while the other 38% were illiterate and provided 22% of the collected data.

We selected 24 key informants among the participants in our survey, precisely 8 shamans (belonging to the Tamang ethnic group), 2 local healers (belonging to the Brahmin ethnic group and experts in Ayurvedic herbal remedies), 14 farmers and plant traders.

Local healers and shamans are expert herbalists, recognised and respected by the local community, treating particular diseases. They are frequently visited by sick people, since modern medical services are scarce in the study area and are often ineffective in treating particular diseases. These healers guided us in their field walk to collect medicinal plants, and allowed us to witness their therapeutic work. As such, it was possible to gather a great deal of information and accurately record it during the course of the study.

In Nepal, farmers are also the custodians of a deep knowledge of the natural environment, the traditional uses of plants, and sustainable management of natural resources (Thorn et al., 2020). Plant traders are familiar with medicinal plants, which make up a significant percentage of their household income, but only shared their knowledge in exchange for a reward (Humagain & Shrestha, 2009).

2.1.Plant diversity

This PhD research reported 318 ethnobotanical uses of 116 taxa belonging to 57 families and 107 genera. The most represented family was Asteraceae with 12 species, followed by Fabaceae (7 species), Lamiaceae (6 species) and Zingiberaceae (5 species). Amaranthaceae, Rutaceae and Poaceae families were represented by 4 species each. The following families were represented by 3 species each: Apocynaceae, Araceae, Euphorbiaceae, Lauraceae, Meliaceae, Urticaceae. Finally, other 43 families were represented by 1 or 2 species each. Herbs were the most representative (36%), followed by trees (27%), shrubs (25%), climbers (8%), parasitic plant species (2%) and ferns (2%).

These data are in accordance with previous studies conducted in nearby zones of Nepal (Bhattarai et al., 2009; Shrestha et al., 2016).

Among the 116 plants documented, 73 plant species were collected from the wild, 29 were cultivated and 14 were naturalised plants. Table 2 lists the plants in alphabetic order, indicating their scientific names, local names (in Nepali, Tamang, Newari, and other languages), family names. For a detailed description of their traditional uses, see Appendix III (Ambu et al., 2020).

Table 2. List of plant species recorded in the study area.

N	***Scientific name*** **Local name(s)**	**Family**
1	*Abrus precatorius* L. Rati geri	Fabaceae
2	*Achyranthes bidentata* Blume Dok, Datiwan, Ghyurupuchu, Ghiughiuru	Amaranthaceae
3	*Acorus calamus* L. Seda	Araceae
4	*Aegle marmelos* (L.) Corrêa Bel	Rutaceae
5	*Agave cantala* (Haw.) Roxb. ex Salm-Dyck Ketuki	Asparagaceae
6	*Albizia julibrissin* Durazz. Shirish	Fabaceae
7	*Asparagus racemosus* Willd. Kurilo	Asparagaceae
8	*Ageratina adenophora* (Spreng.) R.M. King & H. Rob. Banmara	Asteraceae
9	*Aloe vera* (L.) Burm.f. Ghyukumari	Xanthorrhoeaceae
10	*Amaranthus cruentus* L. Latte	Amaranthaceae
11	*Amaranthus hybridus* L. Latte	Amaranthaceae
12	*Amaranthus viridis* L. Gaute bangan	Amaranthaceae
13	*Arenaria benthamii* Fenzl ex Torr. & A.Gray Tangne	Caryophillaceae
14	*Artemisia indica* Willd. Titepati, Dusun	Asteraceae
15	*Bauhinia variegata* L. Koiralo	Fabaceae
16	*Berberis asiatica* Roxb. ex DC. Chutro	Berberidaceae
17	*Bergenia ciliata* (Haw.) Sternb. Pashanved, Bra mindhu	Saxifragaceae
18	*Bidens pilosa* L. Buk tinai	Asteraceae
19	*Blainvillea acmella* (L.) Philipson Saprumo	Asteraceae
20	*Blumea aromatica* DC.	Asteraceae
21	*Boehmeria virgata* (G.Forst.) Guill. subsp. *macrophylla* (Hornem.) Friis & Wilmot-Dear. Chalnesisnu	Urticaceae
22	*Boehmeria rugulosa* Wedd. Bhlan chhing	Urticaceae

23	*Boenninghausenia albiflora* (Hook.) Rchb. ex Meisn. Thangkap mra	Rutaceae
24	*Brassica rapa* L. Sarson	Brassicaceae
25	*Bryophyllum pinnatum* (Lam.) Oken Kidney stone medicine	Crassulaceae
26	*Calotropis gigantea* (L.) Dryand. Arka, Aank, Akh	Apocynaceae
27	*Carica papaya* L. Papaya	Caricaceae
28	*Catharanthus roseus* (L.) G. Don Barhamase phul	Apocynaceae
29	*Cautleya spicata* (Sm.) Baker Pahelo Ausadhi, Jungli haldi	Zingiberaceae
30	*Centella asiatica* (L.) Urb. Tajoimra, Kholachagaian, Kolacha	Apiaceae
31	*Choerospondias axillaris* (Roxb.) B.L. Burtt & A.W.Hill Lapsi	Anacardiaceae
32	*Cinnamomum glanduliferum* (Wall.) Meisn. Tagba	Lauraceae
33	*Cipadessa baccifera* (Roth) Miq. Painati	Meliaceae
34	*Cirsium wallichii* DC. Thakal, Achangpolo, Chwacan	Asteraceae
35	*Citrus x limon* (L.) Osbeck Nibuwa	Rutaceae
36	*Citrus x sinensis* (L.) Osbeck Junar	Rutaceae
37	*Clematis buchananiana* DC. Chyanmangre	Ranunculaceae
38	*Colebrookea oppositifolia* Sm. Dhursil, Busul sul	Lamiaceae
39	*Colocasia esculenta* (L.) Schott Pidalu, Taia	Araceae
40	*Coriaria nepalensis* Wall. Bhujinshin, Hakupaku	Coriariaceae
41	*Crateva religiosa* G. Forst. Siplekan	Capparaceae
42	*Curcuma angustifolia* Roxb. Haldi	Zingiberaceae
43	*Curcuma caesia* Roxb. Mlang haldi	Zingiberaceae
44	*Curcuma longa* L. Haldi	Zingiberaceae
45	*Cuscuta reflexa* Roxb. Sikari lahara, Sky grass	Convolvulaceae
46	*Daphne bholua* Buch.-Ham. ex D.Don Lokta	Thymelaceae
47	*Dioscorea bulbifera* L. Dhingyui mindhu	Dioscoreaceae
48	*Diploknema butyracea* (Roxb.) H.J.Lam Chyuri (N)	Sapotaceae
49	*Drymaria cordata* (L.) Willd. ex Schult. Abijalo,Tangar, Abijal, Abisal ghe, Kai bugain	Caryophillaceae
50	*Duhaldea cappa* (Buch.-Ham. ex D.Don) Pruski & Anderb. Ranaven, Ranabhyang	Asteraceae
51	*Eclipta prostrata* (L.) L. Mashi mra	Asteraceae

52	*Elephantopus scaber* L. Buti jhar, Tinai	Asteraceae
53	*Eulaliopsis binata* (Retz.) C.E.Hubb. Arkhen khar	Poaceae
54	*Euphorbia hirta L.* Rato lahare, Walagughi	Euphorbiaceae
55	*Ficus racemosa* L. Dumri	Moraceae
56	*Ficus semicordata* Buch.-Ham. ex Sm. Ngedhore	Moraceae
57	*Galinsoga parviflora* Cav. Bhuitimur	Asteraceae
58	*Gossypium arboreum* L. Kopi, Kapash	Malvaceae
59	*Hemionitis anceps* (Blanf.) Christenh. Rani sinka	Pteridaceae
60	*Iris domestica* (L.) Goldblatt & Mabb. Darware mindhu	Iridaceae
61	*Jasminum mesnyi* Hance Ghyi fui, Gaiful	Oleaceae
62	*Jatropha curcas* L. Arin, Sajiwan, Mandhar	Euphorbiaceae
63	*Justicia adhatoda* L. Asuro, Basak	Acanthaceae
64	*Lablab purpureus* (L.) Sweet Lahare guki	Fabaceae
65	*Lantana camara* L. Polung	Verbenaceae
66	*Leucas cephalotes* (Roth) Spreng. Topdoi mra	Lamiaceae
67	*Litsea cubeba* (Lour.) Pers. Siltimur	Lauraceae
68	*Machilus odoratissima* Nees Kaulo	Lauraceae
69	*Mangifera indica* L. Aanp	Anacardiaceae
70	*Melia azedarach* L. Bakaino, Bakena	Meliaceae
71	*Mentha spicata* L. Naasun	Lamiaceae
72	*Momordica charantia* L. Karela	Cucurbitaceae
73	*Musa* x *paradisiaca* L. Kera	Musaceae
74	*Myrica esculenta* Buch.-Ham. ex D. Don Kaphal	Myricaceae
75	*Nephrolepis cordifolia* (L.) C. Presl Tui amala	Polypodiaceae
76	*Nyctanthes arbor-tristis* L. Parijat	Oleacaeae
77	*Ocimum tenuiflorum* L. Tulsi	Lamiaceae
78	*Oryza sativa* L. Sun, Nalasun	Poaceae
79	*Osbeckia nepalensis* Hook. Chulsi	Melastomataceae
80	*Oroxylum indicum (*L.) Kurz Tatelo, Tarlason	Bignoniaceae

81	*Osyris lanceolata* Hochst. & Steud. Borsajini	Santalaceae
82	*Oxalis corniculata* L. Pang qui, Nakbruigumba, Pauja gai	Oxalidaceae
83	*Perilla frutescens* (L.) Britton Silam	Lamiaceae
84	*Phaseolus vulgaris* L. Rajama	Fabaceae
85	*Phyllanthus emblica* L. Amala	Phyllanthaceae
86	*Picrasma quassioides* (D.Don) Benn. Nim kath	Simaroubaceae
87	*Piper betle* L. Paan	Piperaceae
88	*Piper retrofractum* Vahl Pan gughi	Piperaceae
89	*Plumbago zeylanica* L. Chitu, Ping chittu	Plumbaginaceae
90	*Pogostemon benghalensis* (Burm.f.) Kuntze Rutula	Lamiaceae
91	*Psidium guajava* L. Amba, Guava	Myrtaceae
92	*Rhaphidophora glauca* (Wall.) Schott Birlahara	Araceae
93	*Ricinus communis* L. Taturoro	Euphorbiaceae
94	*Rubus ellipticus* Sm. Aniselu, Polang	Rosaceae
95	*Saccharum officinarum* L. Ukhu	Poaceae
96	*Saraca asoca* (Roxb.) J.J.de Wilde Ashoka	Fabaceae
97	*Searsia parviflora* (Roxb.) F.A.Barkley Satibro	Anacardiaceae
98	*Senegalia catechu* (L.f.) P.J.H.Hurter & Mabb. Khayar	Fabaceae
99	*Smallanthus sonchifolius* (Poepp.) H. Rob. Bhuishyau	Asteraceae
100	*Solanum nigrum* L. Camai	Solanaceae
101	*Stephania glandulifera* Miers Gundri gano	Menispermaceae
102	*Strobilanthes pentastemonoides* (Nees) T.Anderson Gathe	Acanthaceae
103	*Swertia angustifolia* Buch.-Ham. ex D. Don Chiraito, Kampman	Gentianaceae
104	*Syzygium cumini* (L.) Skeels Jamuna	Myrtaceae
105	*Tagetes erecta* L. Sayapatri, Saipatri sun	Asteraceae
106	*Terminalia bellirica* (Gaertn.) Roxb. Barla	Combretaceae
107	*Tinospora sinensis* (Lour.) Merr. Gurjo	Menispermaceae
108	*Toona hexandra* (Wall.) M.Roem. Tuni	Meliaceae
109	*Urtica dioica* L. Sisnu	Urticaceae

110	*Valeriana hardwickei* Wall. Daling	Caprifoliaceae
111	*Valeriana jatamansi* Jones ex Roxb. Jatamasi, Dhalin	Caprifoliaceae
112	*Viscum articulatum* Burm. f. Khakhre bali	Santalaceae
113	*Woodfordia fruticosa* (L.) Kurz Daduimre, Bhyur ghara	Lythraceae
114	*Wrightia arborea* (Dennst.) Mabb. Glemindhu	Apocynaceae
115	*Zea mays* L. Makai	Poaceae
116	*Zingiber officinale* Roscoe Aduwa	Zingiberaceae

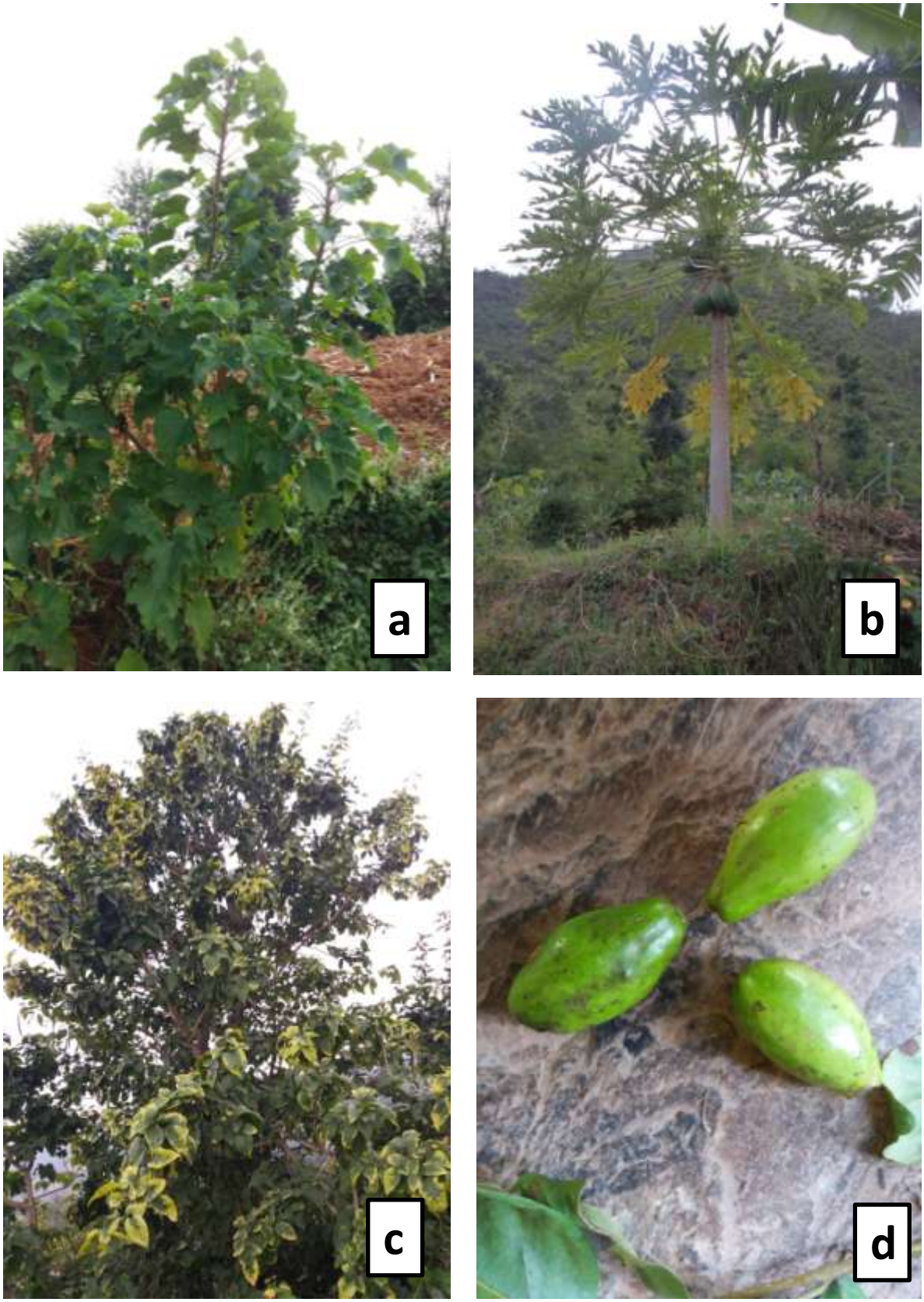

Plate 16. Some trees documented in the present study.: (a) *Jatropha curcas*; (b) *Carica papaya*; (c) *Aegle marmelos*; (d) fruits of *Terminalia bellirica*.

Plate 17. Some plants documented in the present study: (a) *Cuscuta reflexa*; (b) *Perilla frutescens*; (c) *Osbeckia nepalensis*; (d) E*clipta prostrata*.

Plate 18. Outodoor maize storage outside a traditional house.

Plate 19. Woman exposing corn kernels (*Zea mays)* to dry in the sun.

2.2.Local names of plants

We took every care to accurately transcribe the local names of the plants to avoid any misunderstanding, thanks to the help of local people and botanists from RECAST.

Local names are an expression of the cultural substratum of each community and often reflect local knowledge about the habitat, morphology, similarity to some animal, uses and other attributes of plants (Camarda & Guarrera, 2005; Manandhar, 2002).

For example, in Nepali "lahara" refers to climbing plants. The climber *Rhaphidophora glauca* (Wall.) Schott, is known as "Birlahara", which means strong ("bir") climber ("lahara"). "Tite" means bitter and "pat" leaf, so "Titepati" is the local name of *Artemisia* spp., and indicates the bitter taste of the leaf. In the Tamang language, "rana" means ear and "bhyang" sheep. Hence, "Ranabhyang" is the local name given to *Duhaldea cappa* (Buch.-Ham. ex D.Don) Pruski & Anderb, due to the shape of its leaves being similar to a sheep's ears.

2.3.Ethnomedicinal uses of plants

In this PhD research, informants reported 271 citations of medicinal uses for 101 plants of the 116 species recorded. Usually, they selected plants that are easy to find, either growing wild near villages or cultivated in home gardens. The home garden ("Ghar bagaincha" in Nepali) refers to a traditional system of land use around a farm, where different plant species are grown and maintained by members of a community for their own needs (Gautam et al., 2006).

Wild medicinal plants were found along the edges of paths, the banks of rivers and streams, and in cultivated land. Only in a few cases, especially in the case of the shamans, were the medicinal plants collected in inaccessible areas of the forest, such as *Viscum articulatum* Burm. f., *Piper retrofractum* Vahl and *Picrasma quassioides* (D.Don) Benn. There are some species that were found both in nature and in home gardens.

Medicinal plants were reported for the treatment of 79 human diseases and 3 animal disorders. These diseases were grouped into 13 categories related to human diseases and 1 category related to cattle diseases (Table 3).

Table 3. Diseases included in each illness category.

Illness category	Diseases	Number of citations
Fever	Fever, high fever, malarial fever, typhoid fever, "internal" fever (feeling of higher level of heat inside the body)	56
Gastrointestinal	Stomach diseases, gastritis, vomiting, indigestion, loss of appetite, diarrhoea, dysentery, constipation (*kabjiat*), liver stones, abdominal pain, blood stool, flatulence	45
Muscolo-skeletal	Rheumatism, body pain, joint pain, joint trauma, joint swelling, ankle sprains, bone fracture, dislocations and sprains of the limbs	30
Dermatological	Skin diseases, skin infections, cut, wounds, pimples, boils, itchy pustules, burns	25
Antidote	Food and drink poisoning, snake bite	19
Respiratory diseases	Cough, cold, cooling diseases, sinusitis, rhinitis, throat irritations, breathing problems	17
Oral, dental, ENT (ear, nose and throat)	Gums problem, mouth swelling, mouth infections, dry mouth, swelling of the dental glands, toothache, tongue infections, eye diseases, "red eyes" (conjunctivitis), nose swelling, ear infections, earaches	15
Metabolic	Blood purification, jaundice, diabetes, goiter	15
Nervous system	Migraine, headache, anxiety, insomnia	14
Maternal diseases	Difficulty in childbirth, infertility, menstrual cycle disorders, postpartum bleeding	11
Urogenital	Urinary problems, bladder swelling, blood in the urine, kidney stones	9
Cardiovascular diseases	High blood pressure, blood circulation disorders, heart attack, piles	6
General health	Cancer, weakness, fatigue	2
Veterinary	Skin problems in cattle, reduced milk (agalactia) in buffaloes, general weakness	7
Total	**14**	**271**

Most plant species (53) were used to treat more than one disease and the others (48) to treat only one type of disease.

From analysis of the available data, it appeared that the most frequent disease categories treated with medicinal plants were: fever (56 citations), digestive system diseases (45), skeletal and muscular system diseases (30), and skin diseases (25).

These results reflect the epidemiological situation in Nepal, particularly in areas with a higher poverty rate. For example, typhoid fever ("myadhe joro" in Nepalese), caused by the bacillus *Salmonella typhi* and *S. paratyphi*, is a significant cause of morbidity and mortality in Nepal, especially in rural areas, where poor sanitation and lack of drinking water are the cause of many infections and their outbreaks (Andrews et al., 2018; Petersiel et al. 2018) (Figure 3).

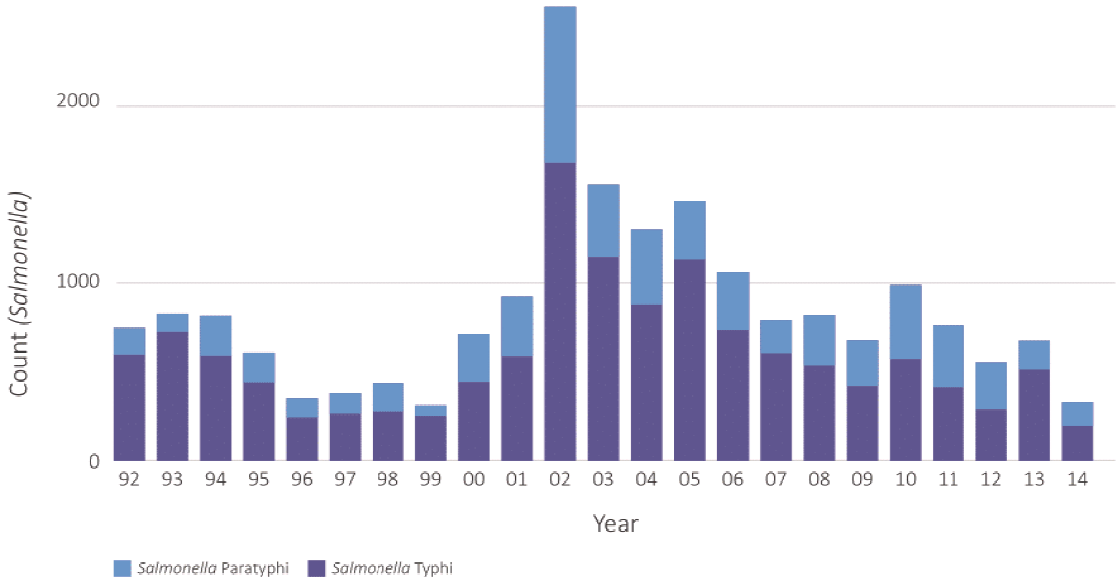

Figure 3. Typhoid and paratyphoid trend from 1992 to 2014 in a densely populated area of Kathmandu (https://www.coalitionagainsttyphoid.org/).

During the monsoon season, the contamination of drinking water with faecal matter becomes a public health problem (Rai et al., 2012) causing other various infectious diseases such as cholera, gastrointestinal infections, influenza, and hepatitis A.

Musculoskeletal diseases also represent a significant health problem in rural areas of Nepal, and is especially common among farmers, whose work is subject to significant effort, risks, and accidents (Mahto & Gautam, 2018). But the lack of orthopaedic care leads to worsening of musculoskeletal pathologies, with a consequent decrease in quality of life (disability, chronic pain etc.) (Chawla et al., 2016).

Skin diseases are one of the most widespread health problems in the hilly and rural communities of Nepal (Karn et al., 2010; Walker et al., 2008): scabies, eczema, fungal and bacterial infections, pruritus, warts, loss of skin colour (vitiligo), urticaria, moles and birthmarks, nodules, cysts, and acne. Some problems, such as cuts, wounds, and burns are related to outdoor activities, such as agriculture and timber cutting. Skin diseases are a serious problem for the population since they cause physical discomfort, they have a long duration and visibility, and can give rise to discrimination due to ancestral fears and beliefs in rural villages (Shrestha et al., 2012).

Overall, despite recent influences from Western medicine, people from ethnic communities continue to rely on traditional medicine. This seems to confirm the therapeutic efficacy of local plants for the treatment of the most common and widespread pathologies (Bechan & Prasad, 2011).

2.4.Herbal Remedies

Herbal remedies for treating different diseases were prepared using various parts of the plant, mainly underground parts (roots, rhizomes, bulbs, and tubers) (about 24%), whole plants (23%), stem/bark and fruits (about 10% respectively), followed by seeds, leaves, flowers, latex, and others.

For the treatment of human diseases, informants reported 98 plant species used in various medicinal preparation forms, based on a single plant or polyherbal formulations, and different routes of administration. The prescribed quantity was always approximate, depending on how the healer judged the severity of the disease. The dosage frequency was described in terms of number of times a day and the duration as number of days or weeks.

Generally, fresh juice was the preferred form of administration due to the simplicity of preparation —- crushing the plant in a stone mortar —- and because this was also an excellent way of getting vitamins and minerals from the plant. The juice had to be taken a short time after preparation. Medical remedies were rarely stored for later use. Some plants were eaten raw after harvesting, especially roots, fruits, seeds, and tubers, or cooked with different ingredients to make a vegetable soup.

Another popular form of preparation for herbal remedies included “poultice”, generally prepared by crushing the plant portions to a pulpy mass, and “compresses”, a piece of cloth soaked in a plant decoction or infusion. Other preparation forms were: direct application, herbal teas (including infusion and decoction), inhalation, ointments, maceration and smoking. The informants generally preferred fresh plants because they found them more effective. Sometimes, dried plants were used when fresh plants were not available, mainly in the form of powders.

Plant parts were generally prepared using hot or cold water as a solvent, but other solvents such as milk, honey, lemon juice, rapeseed oil, “ghee” (clarified butter) were occasionally employed. Single herbs, or multiple herbs combined to produce original recipes, can be used. In particular, a Newar medicinal recipe used to treat various diseases, and prepared by mixing parts of different plant species, was found to be in practice. A typical feature of Newar medicine is that different medicinal plants can be used to treat a number of diseases (Balami, 2004).

Local healers are able to determine the toxicity or otherwise of medicinal plants. Some species, for example, are useful when used externally, but their use internally is toxic. Or the toxicity may depend on the quantity taken: *Iris domestica* (L.) Goldblatt & Mabb. rhizome, when used as medicine, causes abortion if taken in large quantities.

2.5.Other uses of plants

The plants documented in this study had other uses in daily life, such as food, beverages, fermented drinks, roofing, ropes, paper and furniture, or they can be employed for ornamental, social, and religious purposes. Often these plants were grown in home gardens.

For example, *Agave cantala* (Haw.) Roxb. ex Salm-Dyck and *Eulaliopsis binata* (Retz.) C.E. Hubb. were used for traditional roofing of houses. The fibres obtained from the seeds of *Gossypium arboreum* L. were reportedly used in the production of blankets and incense wicks. Some plants were used to prepare alcoholic beverages ("chhaang"), namely *Blumea aromatica* DC., *Clematis buchananiana* DC., and *Oryza sativa* L.. Other species were commonly used in daily nutrition: *Brassica rapa* seeds to make rapeseed oil, the leaves of *Amaranthus* spp. for soups ("satoo") and its seeds for bread ("chapati"). *Carica papaya* L.*, Musa* x *paradisiaca* L., *Choerospondias axillaris* (Roxb.) B.L. Burtt & A.W. Hill, *Citrus* spp. were grown for their edible fruits. *Catharanthus roseus* (L.) G. Don, *Aloe vera* (L.) Burm. f. and *Colebrookea oppositifolia* Sm. were known for their ornamental value.

Many plants have an important religious and spiritual value for the people of the study area. Recently, some researchers have highlighted the strong link between religious culture and medicinal plants (Majupuria, 2009; Niroula & Singh, 2015; Sapkota, 2013). More precisely, the discovery of the therapeutic virtues of plants could have led people to develop religious sentiments towards the plant world. The following table lists in schematic form the main religious uses of some medicinal plants documented in this research and confirmed by the literature (Gupta, 2001; Nath & Mukherjee, 2015; Pelissero, 2016; Shrivastava et al., 2014) (Table 4).

Table 4. List of sacred plants in the study area.

Scientific name	Religious use
Achyranthes bidentata Blume	It is used in various worships and festivals.
Aegle marmelos (L.) Corrêa	Among Newar it is worshipped for Shiva in a ritual called "Ihi", the symbolic marriage of the girls. Wood is burned for sacrificial fires.
Asparagus racemosus Willd.	Placed by Tamang above the threshold of the doors to protect themselves from the evil.
Artemisia indica Willd.	It is offered to Shiva, Vishnu, and Surya. The dried plant is used as incense.
Bauhinia variegata L.	The plant is associated with myths and legends.
Boehmeria rugulosa Wedd.	Wood is used for the production of religious masks.
Brassica rapa L.	Its oil is used for pacifying planets. Among Newar, it is offered to Mercury.
Calotropis gigantea (L.) Dryand.	Sacred plant to sun god or Surya, used in naming ceremony.
Cinnamomum spp.	Product of this plant is used in religious ceremonies.
Citrus spp.	Used during Tihar festival.
Colocasia esculenta (L.) Schott	Leaves of this plant is worshipped in the name of Chamunda.
Colebrookea oppositifolia Sm.	Flowers are sold in urban markets for temple offerings, in January-February when other flowers are scarce.
Curcuma angustifolia Roxb.	It is used in many Hindus ceremonies.
Curcuma longa L.	Used in various religious rituals.
Daphne bholua Buch.-Hamm. ex D. Don	Sacred and offered to Shiva.
Diploknema butyracea (Roxb.) H.J. Lam	Worshipped in occasion of the marriage.
Eclipta prostrata (L.) L.	Plant is used in various ceremonies.
Eulaliopsis binata (Retz.) C.E. Hubb.	Worshipped for Vishnu.
Ficus racemosa L.	Religious tree both for Hindus and Buddhists.
Gossypium arboreum L.	Wicks immersed in oil or ghee and burnt in religious ceremonies are made from cotton.
Jasminum spp.	Used in various Hindus and Buddhist ceremonies.
Leucas cephalotes (Roth) Spreng.	Flowers are offered especially to Ganesh. Newar offer flowers at the crossroads to appease the evil spirits.
Mangifera indica L.	Sacred tree considered a great saviour.
Musa x *paradisiaca* L.	Its fruits are symbol of fecundity.
Nyctanthes arbor-tristis L.	Mythological tree of Indra's Paradise.
Ocimum tenuiflorum L.	Worshipped for Lakshmi, the wife of Vishnu.
Oroxylum indicum (L.) Kurz	Seeds have a strong symbolic meaning in Tamang culture and are used widely in the practices of shamans.
Oryza sativa L.	Sacred plant, used in many Hindus and Buddhist ceremony, and in shamanic healing rituals.
Phyllanthus emblica L.	Sacred tree, worshipped by Hindu women in Kartik (September-October) to obtain male progeny.
Piper betle L:	Its leaves are used in all ceremonies, especially in Tihar.
Psidium guajava L.	Used during Vijaya Dashmi festival.

Rubus ellipticus Sm.	As the plant has thorns, ghosts are believed not to come near the bush. it is used during funerals.
Saccharum officinarum L.	Used during Dashain and Shivaratri festivals.
Saraca asoca (Roxb.) J.J. de Wilde	Tree symbol of immortality.
Senegalia catechu (L.f.) P.J.H. Hurter & Mabb.	Worshipped for Agni, used to make fireworks during festivals.
Syzygium cumini (L.) Skeels	There are several references of this tree in Hinduism and Buddhism.
Tagetes erecta L.	Flowers are offered to goddesses and used for the creation of garlands and decorations during weddings, festivals and other religious events.
Urtica dioica L.	The plant is put on the door to ward off evil spirits.
Valeriana jatamansi Jones ex Roxb.	The plant is used by shamans and in the preparation of incense for religious purposes.
Zea mays L.	In Buddhism, several deities are depicted holding ears of corn.
Zingiber officinale Roscoe	Used in various rituals.

Plate 20. (a-b) Natural source of water supply. (c) A ”dhunge dhara”, a traditional stone drinking fountain in rural area of Kavrepalanchok.

Plate 21. *Eulaliopsis binata* used to cover the roofs of traditional houses.

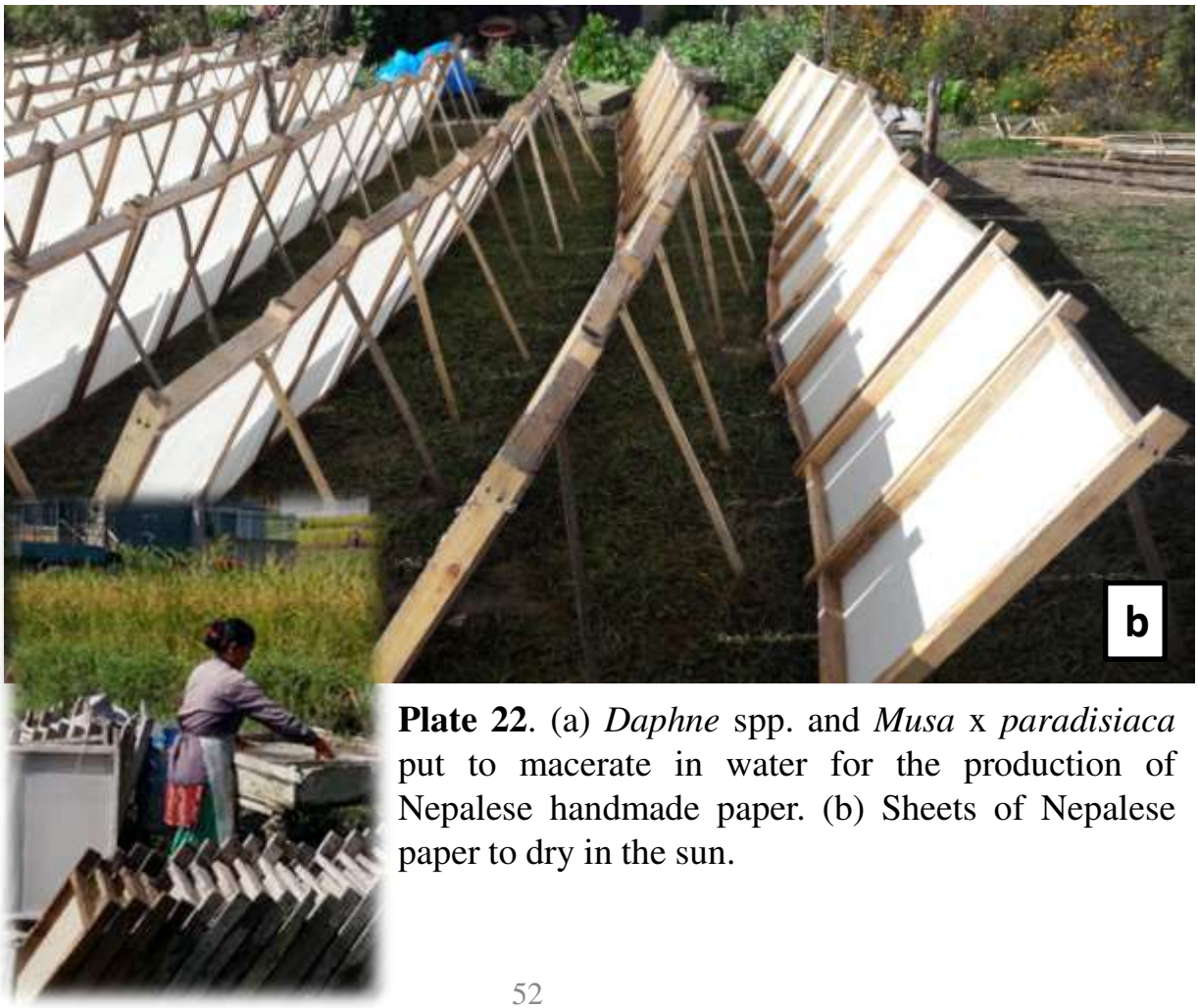

Plate 22. (a) *Daphne* spp. and *Musa* x *paradisiaca* put to macerate in water for the production of Nepalese handmade paper. (b) Sheets of Nepalese paper to dry in the sun.

Plate 23. Roots of *Asparagus racemosus*, used for improve lactation in buffaloes.

c

Plate 24. In Buddhist sacred paintings many trees and plants occur.

(a) Queen Maya, the mother of "Buddha" gives birth to him in a miraculous way, in a grove of *Tectonia grandis* (teak trees).

(b) Lotus flower *(Nelumbo nucifera),* symbol of purity, in a jar representing the prosperity and longevity.

(c) Milarepa, Tibetan yogi of 11th-12th century, surrounded by symbolic trees and flowers, such as top left probably a plant of *Rhododendron arboreum.*

Plate 25. Ritual masks in "teki" wood (*Boehmeria rugulosa*) sold in Kathmandu.

Plate 26. *Ocimum tenuiflorum* grown in pots in the Hindu temple of Shree Kusheswor (Neapalthok).

Plate 27. Every devoted Brahmin plants *Ocimum tenuiflorum* in his gardens, dedicates an altar to it and worships it.

Plate 28. Garlands made with flowers of *Tagetes erecta*.

2.6.Data Analysis

To evaluate the ethnopharmacological importance of each plant species and the degree of agreement among informants, some ethnobotanical indices were calculated.

The informant consensus factor (Fic) was used to show the level of informant agreement about use of plants to treat a subcategory of diseases. Since this factor ranges from 0 to 1, a value close to 1 indicates substantial agreement among informants on the use of a few taxa in the treatment of a subcategory of disease, while a low value closer to 0 indicates that informants disagree on which taxa to use (Table 5).

Table 5. Fic by subcategories of diseases calculated only for a number of use reports ≥ 15.

Disease subcategory	Use reports (Nur)	Number of taxa (Nt)	Fic
Fever	56	29	0.49
Gastrointestinal	45	32	0.29
Dermatological	25	18	0.29
Metabolic	15	11	0.28
Oral dental ENT	15	11	0.28
Skeleto-muscular problems	30	24	0.21
Antidote	20	17	0.16
Respiratory disease	17	15	0.13

The results showed that the greatest agreement (0.49) was found for the fever category, followed by gastrointestinal and dermatological categories (0.29). Intermediate agreement among informants was recorded for metabolic, oral/dental/ENT (0.28) and musculoskeletal (0.21) categories, while the others had a Fic lower than 0.20.

Fidelity level (FL) was used to identify the most preferred species for treating certain ailments. It showed that the most mentioned species were *Calotropis gigantea* (L.) Dryand. (100%) for dermatological diseases, *Drymaria cordata* (L.) Willd. ex Schult. (100%) for fever, and *Mangifera indica* L. and *Wrightia arborea* (Dennst.) Mabb. (100%) for gastrointestinal disorders. *Oxalis corniculata* L. and *Centella asiatica* (L.) Urb. were also reported for the treatment of fever, with FLs of 80% and 75%, respectively. Notably, a FL of 75% was also found for *Curcuma caesia* Roxb. for the treatment of maternal ailments (Table 6).

Table 6. FL value of medicinal plants against a given disease category.

Medicinal plants	Therapeutic category	N_P	N	FL value (%)
Calotropis gigantea (L.) Dryand.	Dermatological	5	5	100
Drymaria cordata (L.) Willd. ex Schult.	Fever	5	5	100
Mangifera indica L.	Gastrointestinal	3	3	100
Wrightia arborea (Dennst.) Mabb.	Gastrointestinal	3	3	100
Oxalis corniculata L.	Fever	4	5	80
Centella asiatica (L.) Urb.	Fever	3	4	75
Curcuma caesia Roxb.	Maternal ailments	3	4	75
Achyranthes bidentata Blume	Maternal ailments	4	9	44
A. bidentata	Fever	3	9	33

To better demonstrate the therapeutic properties of the most mentioned medicinal plants, we reported on the main chemical compounds that are probably involved in their specific curative effects (Table 7), based on recent pharmacological investigations.

Table 7. Main chemical compounds allegedly responsible for the therapeutic effects of medicinal plants with higher FL.

Medicinal plant	Main chemical constituents
Calotropis gigantea (L.) Dryand.	Lupeol present in the latex, with wound healing and antimicrobial properties (Saratha et al., 2011).
Drymaria cordata (L.) Willd. ex Schult.	Saponins and related phytosterols, as well as phenols have been associated with antipyretic activity (Akindele et al., 2012).
Mangifera indica L.	Polyphenols and flavonoids from peel fruit with antinflammatory and antioxidant activities (Kuganesan et al., 2017).
Wrightia arborea (Dennst.) Mabb.	Not known.
Oxalis corniculata L.	Phenolic compounds with antibacterial activity (Raghavendra et al., 2006).
Centella asiatica (L.) Urb.	Asiaticoside (triterpenoid) with antipyretic effects (Wan et al., 2012).
Curcuma caesia Roxb.	Saponins with coagulating properties (Donipati et al., 2015).
Achyranthes bidentata Blume	Saponins with antinflammatory and antioxidant effects and promoting blood circulation (Gao et al., 2003).

Relative frequencies of citations (RFCs) were calculated, and the results are shown in Table 8.

Table 8. RFCs has been reported only for species showing a value ≥ 0,1.

Plant species	**RFCs**
Achyranthes bidentata Blume	0.28
Calotropis gigantea (L.) Dryand.	0.16
Drymaria cordata (L.) Willd. ex Schult.	0.16
Artemisia indica Willd.	0.13
Centella asiatica (L.) Urb.	0.13
Curcuma caesia Roxb.	0.13
Daphne bholua Buch.-Ham. ex D.Don	0.13
Oxalis corniculata L.	0.13

Chapter 3: Discussion

3.1. Traditional knowledge of medicinal plants and their uses

This research revealed the usage of 116 plant species, 101 of which were used to cure 79 types of human diseases and 3 types of animal diseases. This traditional knowledge was more prevalent among the elderly, mainly concerning treatment of the most common ailments. For more specific ailments, people turned to traditional healers and shamans, who were able to select an appropriate therapeutic strategy upon correct diagnosis of the disease. In particular, if the diagnosis revealed a spiritual/psychological cause, the healer treated the patient by suggesting the most suitable herbal remedy. On the other hand, if the ailment had an organic cause, the healer referred the patient to a physician. This finding is in agreement with other studies conducted in Nepal (Peters, 2007; Pham, 2019).

It was noted that younger generations have little knowledge of plants and their medicinal potential. Only recently have some young people in the study area and in other parts of Nepal become more interested in traditional practices on the use of medicinal plants by elders and traditional healers (Malla & Chhetri, 2012; Shrestha & Dhillion, 2003).

3.2. Herbal remedies for humans and cattle

The primary source of herbal remedies are herbs, followed by trees, and shrubs. This result is similar to that reported in previous studies on medicinal plants carried out in Central Nepal (Bhattarai et al., 2009; Shrestha et al., 2016; Shrestha & Dhillion, 2003; Uprety et al., 2010). The preference for herbs as medicines could be related to their greater availability, ease of collection, and ease of preparation. Most of the medicinal plants documented were collected in nature, albeit in the vicinity of the villages. This shows that among those ethnic communities, cultivation of medicinal plants was almost absent. This situation could, in the long term, prove to be detrimental to the conservation of certain plant species, especially if harvested in large quantities.

For preparation of remedies, the preferred parts of the plants were the underground parts (roots, rhizomes, tubers, and bulbs), followed by whole plants. Ghimire et al. (2008) have highlighted that root and whole plant removal has a negative effect on plant growth in nature. These observations need to be carefully considered, since some species documented in this study are threatened with extinction due to over-harvesting, and not just for medicinal purposes (Manandhar, 1990). For example, the *Acacia catechu* (L.f.) Willd., *Choerospondias axillaris* (Roxb.) B.L. Burtt & A.W. Hill and *Oroxylum indicum* (L.) Kurz trees are felled to use their wood as timber, fuel, and fodder.

As regards plants used for livestock, it has been observed that local farmers have rich traditional knowledge in selecting, harvesting, and providing the right type of forage for the nutritional and health needs of cattle (Degen et al., 2010). In fact, each farmer chose forage plants not only based on local and seasonal availability, but also for the health properties of these plants for their livestock, properties such as increasing milk production and promoting growth. The most preferred fodders, besides green crops and straw, were the tree foliages of *Ficus semicordata* Buch.-Ham. ex Sm., *Terminalia bellirica* (Gaertn.) Roxb., *Boehmeria* spp. and *Toona hexandra* (Wall.) M. Roem.. For some households, a good income can be earned by selling *Asparagus racemosus* Willd. locally, since its roots are useful to improve milk production in buffaloes.

In addition to *A. racemosus*, another eleven of the 116 plants mentioned by our informants for the preparation of popular medicinal remedies are sold in the streets of the Kathmandu Valley, and are a valuable source of income for local people (Acharya & Rokaya, 2005): these are *Acorus calamus* L., *Bergenia ciliata* (Haw.) Sternb., *Cautleya spicata* (Sm.) Baker, *Curcuma angustifolia* Roxb., *Osyris lanceolata* Hochst. & Steud., *Phyllanthus emblica* L., *Rubus ellipticus* Sm., *Stephania glandulifera* Miers, *T. bellirica, Tinospora sinensis* (Lour.) Merr. and *Valeriana jatamansi* Jones ex Roxb.. For this reason the collection of, and trade in, these species should be controlled to prevent environmental depletion.

3.3. Traditional concept of diseases

Different concepts of disease were found in the study area. The interpretation of diseases in their causes varied according to the social and educational background of the informants. Normally in rural areas a traditional conception of disease prevailed, according to which its cause is not to be found in an external agent, but in a spiritual one. The disease is the result of a psychophysical imbalance caused by a spiritual disharmony (Chapagai, 2007; Khatry 2011). On the contrary, among young people accustomed to a more urban context, an allopathic conception of diseases prevailed. More frequently the two interpretations coexisted and influenced each other.

An example was the way of interpreting fever, both as a disease and as a symptom quite common in hilly rural areas of Kavrepalanchok District (see Table 3). Local people called the fever “jwaro” and considered it influenced by several natural and spiritual factors, such as stress, fatigue, weakness, hot weather, cold, flu and respiratory diseases, malaria (“aulo”), typhoid (“dokh”), evil spirits, sleeping under certain trees like *Shorea robusta* Gaertn. (“Saal”) etc. (Budhathoki, 2011).

3.4. Mixture of medicinal plants

Herbal remedies were prepared mainly from single species, but sometime also from a mixture of plants. Some traditional healers, especially Tibetan doctors ("amchis"), believe that medicinal plant combinations are more efficient in the treatment of a disease than the single plant (Bhattarai et al., 2010). However, the mixture of plants prepared by "amchis" healers are frequently kept secret, and for this reason we could not record data during the interviews at the Namobuddha Buddhist monastery (Bhattarai et al., 2006).

Even among traditional healers sometimes the ingredients of herbal remedies were not completely revealed, since some of them believed this would cause the remedy to lose its effectiveness. Nevertheless, it was possible to record some original combinations of two or more plants used to treat various diseases.

For example, to cure goiter the dried rhizome powder of *Zingiber officinale* Roscoe is mixed with the *Bauhinia variegata* L. bark powder and water and applied to the throat. *Centella asiatica* (L.) Urb. plant juice is sometimes mixed with the juice of *Drymaria cordata* (L.) Willd. ex Schult. and drunk to cure fever. The crushed plant of *Rubus ellipticus* Sm. is mixed with *Osbeckia nepalensis* Hook. and applied to skin infections.

In the Newar community an original recipe consisting of 9 plants was found to be in practice, including *Artemisia indica* Willd., *C. asiatica*, *D. cordata*, *Mentha spicata* L., *Oroxylum indicum* (L.) Kurz, *Oryza sativa* L., *Oxalis corniculata* L., *Picrasma quassioides* (D.Don) Benn., and *Tagetes erecta* L..

3.5. Ritual plants used by shamans

During the direct participation in the healing rites conducted by ethnic Tamang shamans, it was possible to observe the use of various plants of high religious and spiritual value. The choice of plants varies according to the group to which the shaman belongs and to the area in which he lives. Below are the plants most frequently found in the construction of the altar ("thaan") that constitutes the sacred space where the shaman performs the healing rites (Rysdyk et al., 2019).

Areca catechu L. (Arecaceae) ("Supari"). The seed of this palm, known as betel nut, is traditionally used by Asian farmers as a mild narcotic. Shamans used these seeds as an offering to invite good spirits to enter the sacred space of the altar.

Artemisia indica Willd. (Asteraceae) ("Titepati"). It was used as an offering, to purify the environment, as incense.

Castanopsis spp. (Fagaceae) (“Katus”). The leaves were used as offerings to the deities and to ward off troublesome and negative spirits.

Dendrocalamus spp. (Poaceae) (“Bans”). These bamboos were used in many rites related to the cycles of life and death. Also used to weave baskets (“nanglo”) to place sacred offerings.

Ficus religiosa L. (Moraceae) (“Pipal”). Its leaves were used to make ceremonial dishes (“tapari”). The fibres obtained by beating the bark were used to make ropes protecting homes and fields against evil spirits.

Mangifera indica L. (Anacardiaceae) (“Aanp”). Its leaves were used in association with those of other plants in sacred offerings.

Musa x *paradisiaca* L. (Musaceae) (“Kera”). In shamanic healing rites, the “kera” represented the cosmic tree that unites all the realities of the world. Banana leaves were often placed on the ground as a “pure” covering on which the shaman built his altar.

Neyraudia arundinacea (L.) Henrard (Poaceae) (“Gunring”). With its stems were made sticks (“puki”) used on the shamanic altar to represent the cosmos. These sticks were sometimes inserted into small piles of rice and linked together with cotton thread.

Ocimum tenuiflorum L. (Lamiaceae) (“Tulsi”). It was used for purification and spiritual healing.

Oroxylum indicum (L.) Kurz (Bignoniaceae) (“Tatelo”). The flower and seeds of this plant were considered very sacred and called "flowers of the soul" by shamans. They were used in both healing and shamanic initiation rites.

Oryza sativa L. (Poaceae) (“Sun”). The grains of uncooked rice (“akshyaata”), together with other grains, constituted “sata byu”. They could be used for divination, as a sacred offering or as a substance on which to transfer the patient's disease and thus remove harmful energy.

Machilus odoratissima Nees (Lauraceae) (“Kaulo”). The decoction prepared from the leaves and bark was used to sever a patient’s bonds with ghosts or evil spirits. The liquid was also sprayed on the person's body.

Shorea robusta Gaertner (Dipterocarpaceae) (“Saal”). Its resistant leaves were used to make offering baskets (“tapari”). The tree resin (“saal dhup”) was burned like incense.

Thysalonaena maxima (Roxburgh) Kuntze (Poaceae) (“Amliso, Sarji”). The flowers of this plant were used to produce a broom passed over the body of a sick person to ward off disease or negative influences. This plant was also called “witches’ herb” because it was considered effective against witchcraft and black magic. Shamans also used it to transform ordinary water into healing water (“amrita”).

3.6. Novelty and future perspectives

Ethnomedicinal data documented in this study were compared with that of previous studies conducted among ethnic communities living in the hilly rural areas of Central Nepal (Malla & Chhetri, 2009; Shah & Lamichhane, 2017; Shrestha & Dhillion, 2003; Tamang, 2003; Bhattarai et al., 2009; Joshi et al., 2011; Thapa, 2012; Luitel et al., 2014; Tamang & Sedai, 2016). This comparison revealed some species and unusual medicinal uses of plants that had not been referred to previously. The following table 9 shows new results that are typical of the survey area.

Table 9. New ethnomedicinal uses documented in the study area.

Medicinal Species	**New findings**
Mangifera indica L.	The peel of fruit boiled is used to cure gastrointestinal disorders.
Wrightia arborea (Dennst.) Mabb.	The pod septum is eaten to cure intestinal disorders.
Ficus semicordata Buch.-Ham. ex Sm.	Pieces of copper added to a *F. semicordata*-based preparation is useful for curing bloody diarrhoea.
Picrasma quassioides (D.Don) Benn.	The wood from this tree mixed with 8 other plant ingredients in an original Newar recipe is used to treat various diseases.
Rhaphidophora glauca (Wall.) Schott	The plant juice is given to women to promote pregnancy.
Arenaria benthamii Fenzl ex Torr. & A.Gray	This plant is used to fill pillows in case of colds and coughs.
Strobilanthes pentastemonoides (Nees) T.Anderson	Root juice is taken in the treatment of high fever.
Cipadessa baccifera (Roth) Miq.	The stem juice obtained from branchlets of highly regarded by shamans as a good remedy for counteracting food and drink poisoning, this use had never been previously reported.
Cirsium wallichii DC.	Root juice is useful for urinary problems, weakness, and malarial fever.
Coriaria nepalensis Wall.	Ripe fruits and plant juice of in case of indigestion is unexpected, because the leaves and fruits of many Coriaria species are considered poisonous in Asia.
Achyranthes bidentata Blume	Used by local people to treat various diseases such as fever and maternal ailments. These findings are a novelty, because a previous study on the same area reported only the use of this species for the treatment of urinary ailments.

This still partially unexplored pool of ethnomedicine knowledge could be better documented and validated through future studies on the phytochemical profile and biological activities of these plants. This could lead to the discovery of bioactive constituents to be used as a source of new drugs, and could provide socio-economic and health benefits for the local population.

Plate 29. Fodder carried from the forest.

Plate 30. Bamboo basket to carry fodder and fuel-wood.

Plate 31. Sale of vegetables (a) and fruits (b) in Kathmandu street market. The *Annona squamosa* L. (Annonaceae) fruits (b) are valued for their health and medicinal properties.

Chapter 4: Conclusion

The first part of this PhD research showed that the ethnic community living in rural areas of Kavrepalanchok District still retains rich traditional knowledge of medicinal plants that are an important source for primary health care. In fact, although some allopathic medicines are available in government "health posts", most indigenous peoples rely on traditional local healers and shamans for their primary health care needs. Despite the close relationship of the local population with the natural environment in this area, we have observed that this cultural heritage is at risk. Young people are generally attracted to urban and Western lifestyles, and sometimes do not fully understand the value of traditional knowledge. In this regard, educational programs could be developed to arouse interest in younger generations about knowledge and conservation of medicinal plants.

According to Heinrich et al. (1998), the percentage values of the Fidelity Level are very useful for selecting specific plants for further research in bioactive compounds. Similarly, the plants for which we have found novel therapeutic uses could be subject to pharmacognostic and pharmacologic investigation. In addition, they could be easily cultivated in dismissed agricultural lands, turning them into a source of income and bringing valorisation of the territory. This could be of help to plan bioconservation strategies involving people from the rural municipalities of this area. Recently, crops of plants such as *Ziziphus buddhensis* Bhattarai & M.L. Pathak and *Asparagus racemosus* Willd. have been grown for commercial and medicinal purposes in the Temal region. *Z. buddhensis* is an endemic species whose seeds are cultivated and sold for making Buddhist rosaries, and *A. racemosus* is sought after for its roots, which are very effective for increasing lactation in buffaloes.

These two examples reflect growing global demand for natural products for various purposes — drugs, cosmetics, and nutraceuticals, etc. The revaluation of wild herbs has the potential to contribute to the Nepalese local economy, considering that Nepal is estimated to supply Chinese and Indian industries with 7,000–27,000 tons of medicinal and aromatic plants (MAPs) annually for a total value of over US$30 million (Caporale et al., 2020). However, some MAPs are vulnerable or endangered species due to over-harvesting. Therefore, it has become increasingly evident that there is a need to provide detailed sustainable development strategies for natural resources and for biodiversity preservation. Hence, the Nepalese government in recent years has started a policy of more responsible management of MAPs (Thakur, 2018).

On the one hand, the usage of medicinal plants in the study area needs to be explored and documented before oral traditions are lost forever. On the other hand, the active involvement of

local populations in the valorisation, conservation, and management of medicinal plants will encourage future projects aimed at sustainable development of biological and cultural diversity in these rural areas of Nepal.

Part II

Phytochemical and pharmacognostic analysis of selected ethnomedicinal plants

Introduction

The second part of this PhD project focused on certain species widely used in traditional medicine emerging from the previous ethnobotanical investigation conducted in the field. These are valerian plants mainly used for treating nervous system disorders and whose usage has been documented in the healing rites of shamans living in the rural areas of Kavrepalanchok District, Central Nepal.

Scientific interest was focused on two species of Caprifoliaceae, *Valeriana jatamansi* Jones ex Roxb. and *Nardostachys jatamansi* (D.Don) DC., used in traditional medicine for their sedative and anxiolytic properties in Nepal and many other Asian countries. The pharmacognostic and phytochemical characteristics, and the biological effect of the essential oils of these two species, were compared with those of *Valeriana officinalis* L., whose phytotherapeutic use is widespread all over the world.

1. Religious and ritual plants used in Tamang shamanism

Medicinal and aromatic plants have been used for many therapeutic purposes all over the world since ancient times (Joshi et al., 2016; Petrovska, 2012). Some of these plants contain chemicals with sedative, stimulating and hallucinogenic effects, and are often used in traditional medicine for the treatment of central nervous system (CNS) disorders (Alrashedy & Molina, 2016; Sood & Thakur, 2016).

During the previously mentioned ethnobotanical survey, it was possible to actively participate in various healing rituals conducted by shamans of the Tamang ethnic community and document some interesting uses of plants. The sacred tools of Tamang shamans include, for example, *Musa* x *paradisiacal* L., *Dendrocalamus* spp., the seeds of *Oroxylum indicum* (L.) Kurz,, raw rice grains, the leaves of *Ocimum* spp. and *Artemisia* spp., the roots of *V. jatamansi* and *N. jatamansi*, as well as rosaries made with the seeds of *Elaeocarpus sphaericus* (Gaertn.) K. Schum. and *Sapindus mukorossi* Gaertn., and brooms made with *Thysanolaena maxima* (Roxb.) Kuntze.

As reported also by Staub et al. (2011), ritual plants can be used for various purposes — symbolic, sacred, and decorative. They can be burned as incense in order to stimulate self-awareness, to cause altered states of consciousness (shamanic trance) and to seek communication with deities, spirits, and ancestors.

Among the plants reported, two species belonging to the "Valerianaceae" family (actually, Caprifoliaceae) were selected for further investigation, namely *V. jatamansi* (Vj) and *N. jatamansi*

(Nj). The shamans used the dried rhizomes and roots of these plants, pulverised and burned as incense (Manandhar, 2004), to “purify” the environment from negative spiritual energies and promote a state of relaxation in the patient. These data are in agreement with what has been reported in previous studies and literature regarding the psychoactive properties of these two species (Jha et al., 2012; Nandhini et al., 2018; Rather et al., 2012).

Although many studies have focused on the therapeutic potential of psychoactive compounds of plant origin, only some mechanisms of action have been deeply investigated (Carlini & Maia, 2017; Polya, 2003). For this reason, it was decided to evaluate the biological effects of the essential oils of these two Nepalese species. Comparison was then made with the essential oil of *V. officinalis* (Vo), well known for its sedative properties.

In addition, micromorphological characterisation of the roots/rhizomes of these species was carried out, because they are often fraudulently adulterated with other species, owing to their high economic value (Singh et al., 2011). Moreover, examples of adulterations due to confusion in botanical names between Nj and Vj have also been frequently reported (Dhimana & Bhattacharya, 2020).

Plate 32. Powder incense sold near Buddhist and Hindu places of worship.

Plate 33. Leaves of *Artemisia indica* burned by traditional healers to dispel evil energies during healing rituals.

2. Description of the three species

The three species considered belong to the Caprifoliaceae family (before Valerianaceae), which contains about 200 species, distributed all over the world, and mentioned for their various therapeutic uses. The word "valerian" first appeared in the 9th century in the texts of Indian physicians and could be derived from the Sanskrit word "bala", which means "young, powerful, strength", or from the Latin word "valere", meaning "well-being, health", and refers either to aroma or to the clinical effects attributed to these plants (Bhatt et al., 2012). Caprifoliaceae include several plant species of economic value. Some are used for ornamental purposes; some are edible, medicinal, or used for the extraction of precious essential oils (Pilerood & Prakash, 2013).

2.1.*Valeriana jatamansi* Jones ex Roxb.

Vj is also known as "Indian Valerian" and was called "daling" by Tamang people living in the survey area of this study. This plant is mentioned in a story that may be recited by shamans during healing rituals. The story tells of the mythological birth of the first Tamang shaman, Dunjur Bon. According to the version recorded by Brigitte Steinmann (1998), the verses are as follows:

> "*Sunpåti Bhairungse sangsimam* ("I have offered purifying smoke of rhododendron")
> *Daling syukpase sangsimam* ("I have offered some incense of the 'daling' plant")
> *Namgai Nyima Dawada* ("To the Sun in the heaven")".

Vj is a small perennial herb distributed in the Himalaya region at an altitude of 1500–3000 m. The rhizome is thick and horizontal, whereas the descending roots are fibrous. Rhizomes and roots contain important compounds to which the therapeutic properties are attributed: valepotriates, sesquiterpenoids, valerianannoids, jatamanins, lignans, cryptomeridiol, maaliol, xanthorrhizzol, patchouli alcohol and others (Raina & Negi, 2015; Rather et al., 2012). In Indian systems of medicine, Vj roots have long been used to treat various diseases, and as a substitute for Vo in the treatment of nervous disorders (Xu et al., 2012). The sedative properties are attributed to the presence of valepotriates (Rajkumar et al., 2011).

2.2.*Nardostachys jatamansi* (D.Don) DC.

The beneficial and healthful properties of this plant have been known for centuries in the Ayurvedic, Chinese, Greek, and Arabic medicine systems, as well as in some sacred texts such as the Jewish and Christian Bibles (Ambu et al., 2019; Gupta, 2001).

Nj is a small, perennial herb distributed in the Himalaya at an altitude of about 3000–5000 m. Its cylindrical rhizome is woody and covered with brown fibres that are the remains of the leaf

bases, while the roots penetrate deep in the soil. Nj has been used since ancient times in traditional medicine systems such as Ayurveda, Siddha and Unani to treat a wide range of ailments (Dhimana & Bhattacharya, 2020). Nj is considered to be a psychoactive plant (Alrashedy & Molina, 2016). The rhizomes and roots of Nj are mainly used for the treatment of various neurological disorders such as hysteria, epilepsy and mental weakness (Razack et al., 2018). Rhizomes and roots contain many bioactive compounds such as sesquiterpene, coumarins, lignans, neolignans, alkaloids, among which the most active constituents are sesquiterpenes and coumarins (Kamini & Raina, 2013).

Due to over-exploitation of rhizomes for medicinal use, Nj is considered to be an endangered species registered in CITES (Convention on International Trade in Endangered Species of Wild Flora and Fauna) and the Red Data Book of Indian Plants. However, correct evaluation of its conservation status is hindered by confusion between, and adulteration of, the rhizomes with those of *V. jatamansi*, *Selinum candollei* Edgew. and *Selinum vaginatum* C.B. Clarke (Gautam & Raina, 2016; Mabberley & Noltie, 2014).

2.3.*Valeriana officinalis* L.

Vo is a perennial herb common in North America, Europe, and Asia. Its rhizome is short and stoloniferous. Its roots are fibrous, and when they are dry they have a characteristic, unpleasant smell (Nandhini et al., 2018).

Studies on Vo root extracts have revealed many chemical compounds with a broad spectrum of biological activities on the CNS — compounds such as sesquiterpenes (valerenic acid and derivatives), valepotriates, flavonoids, triterpenes, lignans, alkaloids, amino acids such as c-aminobutyric acid and γ-aminobutyric acid (GABA), and quinolinic acid with antioxidant and lipid peroxidation-decreasing effects (Nandhini et al., 2018; Yoo et al., 2015).

In traditional medicine, Vo has been known and used since ancient times as a diuretic, analgesic and cough medicine. In the mid-18th century, it was noted that it had a sedative effects on the nervous system. For this reason, Vo rhizomes, roots and stolons have been used for the treatment of neurotonic states, especially anxiety and sleep disorders, but also neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease (Del Fresno & Carrettero Accame, 2001; Hadley & Petry, 2003; Jung et al., 2014).

3. The essential oils

Essential oils (EOs) and extracts of Vo, Vj and Nj are used in the flavouring, pharmaceutical and fragrance industries (Bhatt, 2012; Taibi et al., 2007). Recently, the chemical composition of these species were investigated (Verma et al., 2011) and the EOs were reported to contain several

monoterpenoids and sesquiterpenoids, to which their pharmacological effects are mainly attributable at the CNS level (Bhatt, 2012; Komori et al., 2006; Saroya & Singh, 2018). Indeed, some of these compounds have been shown to have direct activity on the amygdala region, or inhibit the enzyme-induced breakdown of GABA in the brain, resulting in sedation (Houghton, 1999). However, the mechanisms that underlie the neuroactive effects of Valerian EOs are still poorly investigated.

In the past two decades, studies on EOs have focused not only on their pharmacological properties, but also on problems related to quality control and standardisation of valerian preparations (Samaneh et al., 2010). In addition, EO composition and variation are significantly influenced by the growth conditions of the plants and, of course, by their genetic features (Bhatt, 2012; Rajkumar et al., 2011; Singh et al., 2015). Considering this and the growing interest in these species, this study was aimed at systematically investigating the botanical and genetic features of these plants via micromorphological and DNA barcoding analyses and subsequently, characterising and comparing the chemical composition and neuroactive effects of their EOs, isolated by steam distillation from the root/rhizome of Vo, Vj and Nj.

Chapter 1: Materials and methods

Phytochemical and pharmacognostic investigation on the selected plants involved several steps: finding, identifying and preparing the plant material for subsequent analysis. Chemicals were purchased from Sigma-Aldrich (Milan, Italy), Extrasynthese (Genay, France) and Merck (Darmstadt, Germany).

All details relating to the techniques and procedures used are reported in Cornara et al. (2020) (Figure 4).

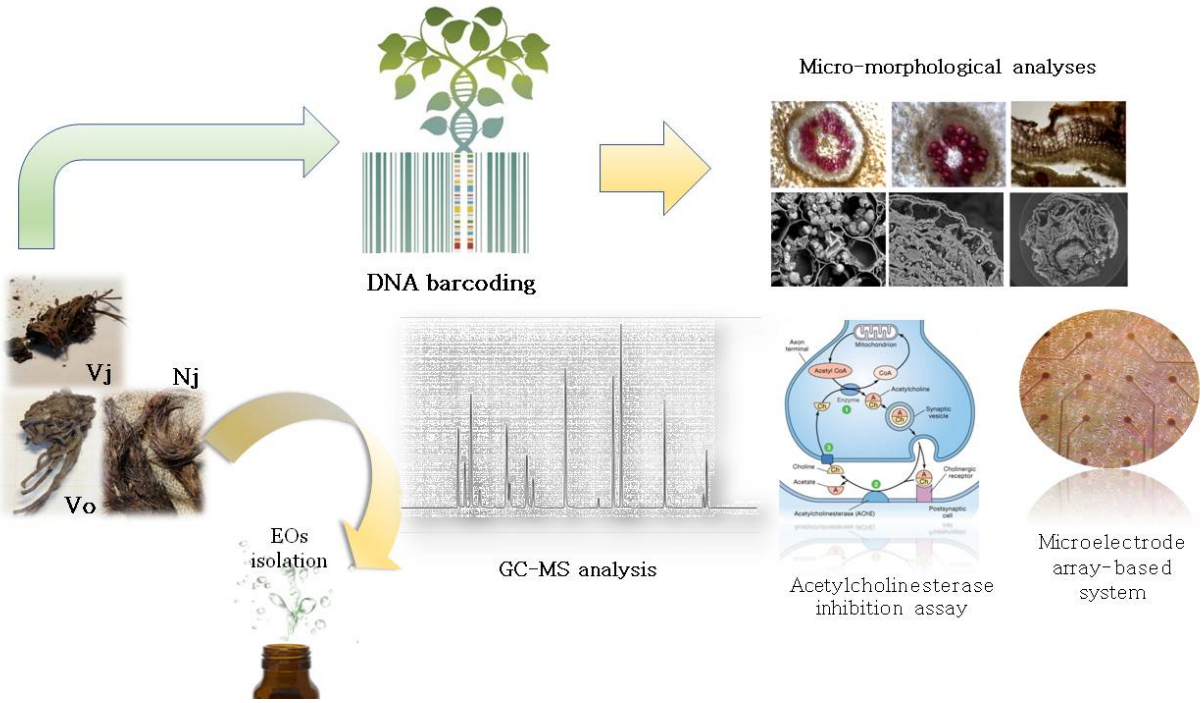

Figure 4. The aim of this PhD second part was to investigate the genetic and botanical features of Vj, Nj and Vo by micromorphological and DNA barcoding analyses and subsequently, to characterize and compare the chemical composition and neuroactive effects of EOs isolated by steam distillation from root/rhizome of the three Caprifoliaceae species (Smeriglio et al., IECPS 2020).

1.1.Plant material and essential oil preparation

The wild rhizomes and roots of Vj and Nj were collected in October 2019 in Darchula District, Western Nepal, and identified by the Nepalese botanist Dr. Dipesh Pyakurel. Voucher specimens were deposited at Tribhuvan University Central Herbarium (TUCH) in Nepal. Rhizomes and roots from Vj and Nj were air-dried, chopped (100 g) and steam-distilled in loco by Dr. Govinda Ghimire of the Nepal Herbs and Herbal Products Association (NEHHPA), until no significant increase in the EO volume was observed (4 h). The EOs were dehydrated on Na2SO4 and stored in burnished vials with nitrogen headspace at 4 ◦C until analysis. The EO yields, calculated on the fresh-weight basis

(% v/w) were 1–1.5% and 1–1.2% for Vj and Nj, respectively. Vo root and rhizome EO derived from plants of Chinese origin was provided commercially by Bios Line S.p.A. (Padua, Italy).

1.2.Species Identification

In the production and trade of herbal medicines, there is often random replacement and/or adulteration of plant species with similar ones (Singh et al., 2011). This not only leads to decrease in the efficacy of the herbal drug but can also, in some cases, be very dangerous if the substitute or adulterant species is toxic (Srirama et al., 2017). Considering this, certain authentication methods were adopted to investigate the selected plant species. Starting from the available plant material, micromorphological characterisations and DNA barcoding were carried out in the DISTAV and UNIMI laboratories respectively.

1.2.1. Micromorphological characterisation

Specimens of roots and rhizomes from the three Caprifoliaceae species were studied with light microscopy and scanning electron microscopy (SEM) analysis, following standard methods for the authentication of botanicals (Joshi & Khan, 2005).

The light microscopy investigation of plant sections and powdered material was carried out directly and after phloroglucinol-HCl staining.

SEM analysis was carried out on samples appropriately fixed and prepared according to the approach of Chieco et al. (2013): dehydrated, hand sectioned, mounted on stubs and coated with 10 nm gold. For the observation, a Vega3 Tescan LMU SEM (Tescan USA Inc., Cranberry Twp, PA, USA) was used at an accelerating voltage of 20 kV.

1.2.2. DNA Barcoding

“DNA barcoding” is a recently developed technique based on DNA markers that allows the identification of biological samples via short DNA sequences. It has proven to be of paramount importance for the authentication of medicinal plant samples (Nithaniyal et al., 2016; Techen et al., 2014). The procedure for the DNA barcoding of Vj, Nj and Vo consisted of the following main steps:

(a) DNA extraction from dried plant material using the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) and the CTAB (cetyl trimethylammonium bromide) method (Doyle, 1991), checking for DNA concentration using a Qubit 2.0 Fluorometer and Qubit dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA, USA).

(b) Choice of the primers and the psbA-trnH intergenic spacer as a DNA barcoding region, in accordance with the approach of Frigerio et al. (2019a).

(c) Polymerase Chain Reaction (PCR) amplification using PuReTaq Ready-To-Go PCR beads (GE Healthcare Life Sciences, Italy) and PCR cycles consisting of steps for template denaturation, primer annealing and primer extension, in accordance with the approach of Frigerio et al. (2019b).

(d) Evaluation by electrophoresis on agarose gel; and purification and sequencing of targeted sequences (amplicons) using an ABI 3730XL automated sequencing machine at Eurofins Genomics (Ebersberg, Germany).

(e) Identification of edited sequences by standard comparison against a GenBank database using the Basic Local Alignment Search Tool (BLAST)[1]. Each barcode sequence was taxonomically assigned to the plant species with the nearest matches, in accordance with the approach of Bruni et al. (2015).

1.3.Phytochemical analyses

The chemical composition of the root and rhizome EOs of the three Caprifoliaceae species (Vj, Nj and Vo) was investigated by gas chromatography-tandem with flame ionisation (GC-FID) and with mass spectrometry (GC-MS), (Agilent Technologies Santa Clara, CA, USA), thanks to collaboration with the UNIME team.

The separation was carried out using an HP-5MS capillary column (30 mm, 0.25 mm coated with 5% phenyl methyl silicone, 95% dimethyl polysiloxane, 0.25 μm film thickness) and helium as carrier gas (1 mL/min). One microliter of 10% EO/CH2Cl2 v/v solution was injected in split mode (100:1) setting the injector and detector temperature at 250 and 280 ◦C, respectively. Elution was carried out according to the following program: 60 ◦C for 6 min, increased to 270 ◦C at 3 ◦C/min and held at 270 ◦C for 4 min (Caputo et al., 2020).

Gas chromatography-mass spectrometry (GC-MS) analysis was carried out on the same instrument, coupled with a mass detector (5975C), by using the same column and operative conditions reported above for analytical GC, but setting the ionization voltage, the electron multiplier and the ion source temperature at 70 eV, 900 V and 230 ◦C, respectively. EO constituents were identified by comparison of the GC retention index with those of C7-C40 n-alkanes, with literature data (Adams, 2017), matching the mass spectral data with MS library NIST 08 (www.nist.gov, accessed on 1 March 2020), by comparison of MS fragmentation patterns with those reported in the literature, and by co-injection with commercially available terpene standards.

[1] https://blast.ncbi.nlm.nih.gov/.

Quantification was carried out by extrapolation of the compound's peak areas from GC-FID profiles.

1.4.Biological activity of EOs

Vj, Nj and Vo are traditionally recognised as psychoactive plants. Despite growing clinical evidence to substantiate this activity, there is little evidence about their pharmacological mode of action (Gramowski et al., 2006). Therefore, for this PhD project, the biological activity of EOs was tested via acetylcholinesterase inhibition assay and microelectrode array analysis. These analyses were carried out in collaboration with the UNIME team and ETT S.p.a, Genova.

1.4.1. Acetylcholinesterase inhibition assay

Acetylcholine is an important neurotransmitter of CNS and Peripheral Nervous System (PNS), whose amount at the synaptic terminations level is regulated by the acetylcholinesterase enzyme (AChE). Recently, some researchers have identified a relationship between some neurological diseases, such as Alzheimer's disease and Parkinson's disease, and cholinergic deficit (Tata et al., 2014; Hampel et al., 2018; Perez-Lloret & Barrantes, 2016).

Considering this, the AChE inhibition ability of the three Caprifoliaceae EOs were evaluated in accordance with the approach of Smeriglio et al. (2018), using galantamine as a reference compound, an alkaloid originally isolated from the Caucasian snowdrop (*Galanthus* spp.) which is well known for its AChE inhibitory activity (Wilkinson, 2001). The results were expressed as an AChE inhibition percentage.

1.4.2. Microelectrode array analysis

In vitro microelectrode array (MEA) is a powerful tool for investigating the neuroactive effects of pure compounds or plant complexes on cultured neural networks (Nam & Wheeler, 2011). In this research, MEA analysis was conducted in order to investigate the effects of the EOs on the CNS, using primary mouse cortical neuronal cultures directly grown on microchips.

Briefly, dissociated neurons, obtained from the cerebral cortices of C57BL/6 embryonic day-15 mice, and properly treated, were left to settle in the centre of each glass 60-electrode MEA chip (60MEA200/30iR-Ti-gr) at Multichannel Systems GmbH (Reutlingen, Germany). Before plating the cells on an MEA chip, the surface was coated with 0.1% polyethylenimine to enhance cell attachment and growth.

Data relating to spontaneous neuronal activity, and resulting from treatment with increasing concentrations of the EOs, was recorded *in vitro* from the 3rd to the 5th week using the USB MEA

120 INV 2 BC System (MCS GmbH, Reutlingen, Germany) and the MC_Rack software (MCS GmbH, Version 4.4.1.0).

Data analysis was carried out using the NeuroExplorer software (Blackrock Microsystems, Salt Lake City,UT, USA) and the results were expressed as half-maximal inhibitory concentrations (IC_{50}, µg/mL).

1.5.Statistical analysis

To find out if the results of this part of the PhD research are significant, one-way analysis of variance (one-way ANOVA) was used. Tukey's test was applied to *in vitro* cell-free based assays and phytochemical analyses, and the Holm-Sidak test was applied to cell-based assays using the SigmaPlot 12.0 software (Systat Software Inc. San Jose, CA, USA).

Moreover, agglomerative hierarchical clustering analysis (AHC) was carried out to highlight phytochemical similarities and dissimilarities among the different plant species investigated.

Chapter 2: Results

The results of this research are summarised below. Further details can be found in Cornara et al. (2020).

2.1. Macro- and micro- morphological characterisation

Macro- and microscopic observation of dried rhizomes, powder drugs, and the transversal sections of roots and rhizomes was performed using light optical and scanning electron microscopes (SEM). The typical characteristics of Vj, Nj and Vo were highlighted, allowing us to distinguish the three species from one other. Table 10 shows the most distinctive elements observed (see Appendix IV, Cornara et al., 2020).

Table 10. Summary table of comparative macro- and micro- characteristics of dried rhizomes of Vj, Nj and Vo.

	Vj	Vo	Nj
Macroscopic characters			
Color and shape	Brownish-black; covered with many brown rootlets	Dull brown; conical with many long stout roots attached	Brownish; elongated, completely covered with silky reddish fibers that are the remains of the leaf bases
Taste	Bitter, camphoraceous	Slightly bitter, spicy	Slightly bitter
Odour	Aromatic like isovaleric acid	Penetrating, resembling valeric acid and camphor	Strongly aromatic
Microscopic characters (light microscope)			
Powder			
Color	Dull brownish	Light brown	Brown
Description	Cork cells in surface view, medullary rays, fragments of fibers, scalariform vessels, starch grains, cells filled with brownish contents	Numerous fragments of parenchyma with rounded or elongated cells containing numerous starch grains; cells containing light-brown resin, rectangular sclereids with pitted walls, xylem isolated or in non-compact bundles, root hairs, cork fragments	Root hairs, medullary rays, cells with reddish-brown contents, cork cells in surface view, fibers, small starch grains, xylem vessels with pitted and scalariform secondary wall thickenings
Transversal section			
Root			
Cortex	Large, multi-layers	Multi-layers of spheroidal parenchymatous cells	
Endodermis		Monolayer; cells suberized and tangentially elongated	
Hypodermis	Abundant oil droplets	Large polygonal cells, containing oil droplets	
Pith	Parenchymatous	Surrounded by a ring of phloem and xylem	
Starch	Abundant in cortex cells and hypodermis	Simple or compound, abundant in cortex cells	
Rhizome			
Cork	Suberized cells with oil droplets		Multi-layers of suberized cells with many oil droplets. Under the cork, many bundles of sclerenchymatous fibers. Inter xylary cork is present
Cortex	Parenchymatous with starch grains, oil droplets and a yellowish-brown substance		
Endodermis	Monolayered	Dark brown, surrounded by a broad cortex consisting of spheroidal parenchyma cells with a large amount of starch	
Pith	Broad parenchymatous with starch grains	Wide and composed of large parenchyma cells, occasionally showing sclereids	In the older portion, pith became necrotic and its cavity appears surrounded by medullary cork layers
Vascular bundles	Collateral, arranged in a circular ring	Numerous, circularly arranged	Collateral, arranged in a circular ring, encircled by cork rings, enclosing a large pith star-shaped

SEM analysis			
Root	Epidermis with numerous root hairs; the cells of the parenchymatous cortex are filled with single starch grains, occasionally compound	Epidermis with many root hairs and small suberized cells; hypodermis made up of polygonal-quadratic cells; starch grains are simple or compound.	
Rhizome	Lobed in outline and showing many vascular bundles circularly arranged around the medullar pith	Regular in outline; internally a circular ring of vascular bundles surrounds the central pith	Many bundles of sclerenchymatous fibers can be observed

2.2. DNA-barcoding

DNA barcoding performed by the University of Milano Bicocca made it possible to confirm the identity of the three Caprifoliaceae species. Table 11 shows declared species, resulted species, origin, collection year and accession numbers inserted into the European Molecular Biology Laboratory (EMBL) Nucleotide Sequence Database[1] (https://www.ebi.ac.uk/ena/browser/). All the samples returned 100% maximum identity (with 100% query coverage).

Table 11. DNA barcoding results.

Declared Species	Resulted Species	Origin	Collection Year	Accession Number
V. officinalis	*V. officinalis*	China	2019	LR861814
V. jatamansi	*V. jatamansi*	Darchula District, Nepal	2019	LR861815
N. jatamansi	*N. jatamansi*	Darchula District, Nepal	2019	LR861816

All the samples returned 100% maximum identity (with 100% query coverage).

2.3. Phytochemical characterisation

Gas chromatography and gas chromatography-mass spectrometry analysis allowed us to track the phytochemical profile of the three plant species investigated, as reported in Table 12.

[1] https://www.ebi.ac.uk/ena/browser/.

Table 12. Chemical composition of *Valeriana officinalis*, *Valeriana jatamansi* and *Nardostachys jatamansi* essential oils. Results are expressed as mean area percentage (%) ± standard deviation (S.D.) of three independent determinations in triplicate (n=3) (Cornara et al., 2020).

Compound	*V. officinalis*	*V. jatamansi*	*N. jatamansi*	KI[a]	Identification[b]
2-Acetylfuran	-	0.50 ± 0.03		912	1,2
Tricyclene	0.14 ± 0.01	-	t	926	1,2
α-Thujene	0.03 ± 0.00	-	0.06 ± 0.00	930	1,2
α-Pinene	3.39 ± 0.15	0.20 ± 0.01	1.59 ± 0.05	939	1,2,3
3-methyl-Valeric acid	-	0.09 ± 0.00	-	945	1,2
α-Fenchene	-	-	0.01 ± 0.00	952	1,2
Camphene	13.85 ± 0.88	0.29 ± 0.01	0.03 ± 0.00	954	1,2,3
Sabinene	0.16 ± 0.01	-	-	975	1,2
β-Thujene	-	-	0.03 ± 0.00	976	1,2
β-Pinene	2.76 ± 0.12	-	2.73 ± 0.13	979	1,2,3
α-Phellandrene	-	-	0.01 ± 0.00	1002	1,2
α-Terpinene	0.02 ± 0.00	-	0.04 ± 0.00	1017	1,2
p-Cymene	0.32 ± 0.01	0.11 ± 0.01	0.08 ±0.00	1023	1,2
Limonene	1.50 ± 0.04	0.07 ± 0.00	0.21 ± 0.01	1029	1,2,3
1,8-Cineole	0.02 ± 0.00	0.02 ± 0.00	0.18 ± 0.01	1031	1,2,3
γ-Terpinene	0.15 ± 0.01	-	0.07 ± 0.00	1059	1,2
Terpinolene	0.05 ± 0.00	-	0.05 ± 0.00	1088	1,2
2-Nonanone	-		0.01 ± 0.00	1090	1,2
Isoamylvalerate	-	-	0.01 ± 0.00	1081	1,2
Linalool	-	-	0.02 ± 0.00	1096	1,2,3
Fenchol	-	-	0.01 ± 0.00	1116	1,2
Camphor	0.07 ± 0.00	-	-	1146	1,2
Borneol	0.87 ± 0.03	0.17 ± 0.01	0.02 ± 0.00	1169	1,2,3
cis-3-Pinanone	-	-	0.03 ± 0.00	1174	1,2
Terpinen-4-ol	0.18 ± 0.01	-	0.14 ± 0.01	1177	1,2,3
α-Terpineol	0.13 ± 0.01	-	0.15 ± 0.01	1188	1,2
Myrtenol	0.28 ± 0.02	-	0.09 ± 0.00	1195	1,2
Citronellol	0.61 ± 0.03	-	-	1225	1,2,3
Thymol, methyl ether	0.30 ± 0.01	0.05 ± 0.00	0.11 ± 0.01	1235	1,2
Carvacrol, methyl ether	-	-	0.36 ± 0.02	1244	1,2

Furfurylvalerate	-	0.14 ± 0.01		1271	1,2
Bornyl acetate	46.90 ± 1.24	0.42 ± 0.02	0.02 ± 0.00	1285	1,2,3
Undecan-2-one	-	-	0.69 ± 0.03	1294	1,2
1,2,3-Trimethylindene	-	-	0.06 ± 0.00	1299	1,2
Mirtenyl acetate	3.94 ± 0.22	-	0.40 ± 0.02	1326	1,2
δ-Elemene	-	-	0.19 ± 0.01	1338	1,2
α-Cubebene	-	-	0.09 ± 0.00	1351	1,2
Cyclosativene	-	-	0.03 ± 0.00	1371	1,2
α-Copaene	-	-	1.90 ± 0.14	1376	1,2
β-Patchoulene	-	6.02 ± 0.32	0.90 ± 0.04	1380	1,2
β-Cubebene	-	-	0.35 ± 0.02	1386	1,2
β-Elemene	1.30 ± 0.05	0.55± 0.02	1.54 ± 0.06	1389	1,2
Cyperene	-		3.42 ± 0.24	1398	1,2
β-Longipinene	-	1.11 ± 0.04	-	1400	1,2
α-Gurjunene	1.13 ± 0.04	-	-	1409	1,2
β-Maaliene	-	-	2.07 ± 0.18	1415	1,2
α-Santalene	-	3.35 ± 0.18	-	1417	1,2
9-Aristolene	-	-	1.42 ± 0.05	1418	1,2
(E)-β-Caryophyllene	0.30 ± 0.02	0.37 ± 0.02	2.94 ± 0.22	1419	1,2,3
Calarene	0.17 ± 0.01	10.57 ± 0.05	8.22 ± 0.44	1433	1,2
α-trans-Bergamotene	-	0.93 ± 0.04	-	1434	1,2
α-Guaiene	-	2.58 ± 0.14	0.05 ± 0.00	1439	1,2
Aromadendrene	-	6.43 ± 0.35	4.17 ± 0.26	1441	1,2,3
Seychellene	-	4.27 ± 0.28	6.90 ± 0.38	1446	1,2
α-Humulene	0.32 ± 0.01	1.29 ± 0.05	0.82 ± 0.04	1453	1,2,3
α-Patchoulene	-	3.35 ± 0.16	0.51 ± 0.02	1456	1,2
allo-Aromadendrene	1.21 ± 0.04	-	-	1458	1,2
γ-Gurjunene	-	11.88 ± 0.48	6.36 ± 0.25	1477	1,2
ar-Curcumene	-	-	1.07 ± 0.03	1479	1,2
Germacrene D	0.05 ± 0.00	-	0.50 ± 0.02	1481	1,2
(E)-β-Ionone	0.14 ± 0.01	-	0.19 ± 0.01	1488	1,2
β-Selinene	-	-	2.55 ± 0.11	1490	1,2
Valencene	-	0.19 ± 0.01	8.05 ± 0.37	1496	1,2
Bicyclogermacrene	0.55 ± 0.02	-	-	1500	1,2

γ-Patchoulene	-	0.76 ± 0.03	-	1502	1,2
α-Bulnesene	-	-	0.26 ± 0.02	1510	1,2
γ-Cadinene	-	-	0.07 ± 0.00	1513	1,2
δ-Guaiene	-	8.53 ± 0.24	-	1516	1,2
α-Maaliene	-	-	1.27 ± 0.06	1522	1,2
δ-Cadinene	-	0.07 ± 0.00	-	1523	1,2
Zonarene	-	-	1.77 ± 0.07	1529	1,2
Kessane	-	2.51 ± 0.12	-	1530	1,2
α-Cadinene	0.50 ± 0.03	0.35 ±0.01	0.86 ± 0.04	1538	1,2
Elemol	-	1.08 ± 0.03	-	1549	1,2
Norpatchoulenol	-	8.06 ± 0.25	-	1555	1,2
GermacreneB	0.31 ± 0.01	-	-	1561	1,2
Maaliol	-	17.43 ± 0.56	-	1567	1,2
α-Vatirenene	-	-	3.07 ± 0.21	1570	1,2
Spathulenol	0.84 ± 0.03	1.32 ± 0.04	0.52 ± 0.02	1577	1,2
Spirojatamol	-	-	3.49 ± 0.18	1585	1,2
Globulol	0.84 ± 0.04	0.45 ± 0.02	0.48 ± 0.03	1590	1,2
Viridiflorol	-	0.16 ± 0.01	1.88 ± 0.08	1592	1,2
Guaiol	1.11 ± 0.02	0.57 ± 0.02	-	1600	1,2,3
Ledol	-	-	0.11 ± 0.01	1602	1,2
Hinesol	0.28 ± 0.01	-	-	1641	1,2
τ-Muurolol	-	-	1.24 ± 0.10	1642	1,2
Valeranol	0.56 ± 0.02	0.97 ± 0.03	-	1652	1,2
α-Cadinol	-	-	2.24 ± 0.20	1654	1,2
Patchoulol	-	0.41 ± 0.02	-	1658	1,2
Jatamansone	-	-	13.96 ± 0.74	1675	1,2
Valeranal	-	-	4.39 ± 0.25	1706	1,2
Aristolone	-	-	2.44 ± 0.13	1763	1,2
Nootkatone	-	-	0.44 ± 0.02	1806	1,2
trans-Valerenyl acetate	13.18 ± 0.55	-	-	1867	1,2
cis-Valerenylisovalerate	1.53 ± 0.04	-	-	2052	1,2

[a]Linear retention index on a HP-5MS column; [b]Identification method: 1 = linear retention index; 2 = identification based on the comparison of mass spectra; 3 = Co-injection with standard compounds; t = traces, less than 0.01%.

From EO comparison, it turned out that Nj had the most complex chemical profile, followed by Vj and Vo. Sesquiterpene hydrocarbons were the most abundant compounds identified in both Nj and Vj, followed by oxygenated sesquiterpenes. However, the main difference found in the phytochemical profile of Nj compared to Vj was the higher percentage of monoterpenes, as well as the presence of other compounds (aldehydes and ketones) which were completely lacking in the Vj EO. Vo showed a completely different phytochemical profile from the other EOs, with oxygenated monoterpenes being the most abundant class, followed by monoterpene hydrocarbons and other compounds, and an almost superimposable amount of sesquiterpene hydrocarbons and oxygenated sesquiterpenes. However, beyond the different classes of compounds identified, the three EOs showed substantial differences in the most representative compounds as well as in their relative abundance (Figure 5).

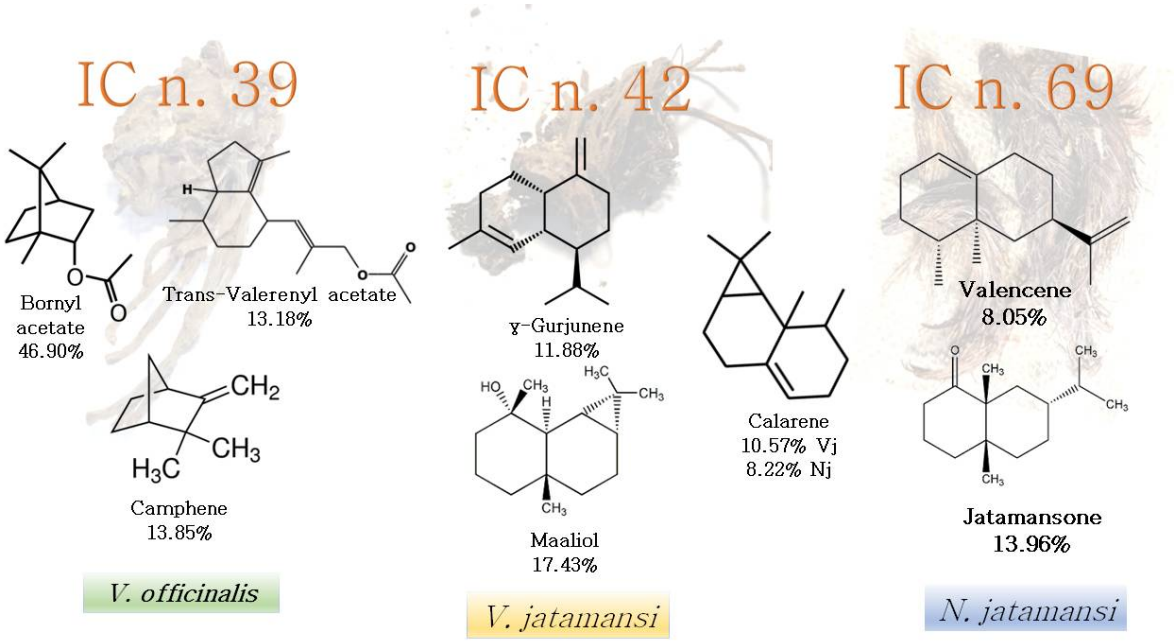

Figure 5. Phytochemical profile. The most representative compounds (Smeriglio, IECPS 2020).

Jatamansone was the most abundant compounds identified in the EO of Nj, along with other substances in smaller quantities such as calarene, valencene, seychellene, gamma-gurjunene and aromadendrene. The most abundant compound identified in the EO of Vj was maaliol, followed by a lower percentage of gamma-gurjunene, calarene, delta-guaiene, aromadendrene and beta-patchoulene. Finally, bornyl acetate was the most abundant compounds in the EO of Vo, along with small quantities of camphene, trans-valerenyl acetate, mirtenyl acetate, alfa-pinene and beta-pinene.

The results of agglomerative hierarchical clustering analyses performed on the 95 compounds identified in the three EOs of the Caprifoliaceae species investigated are reported in Figure 6.

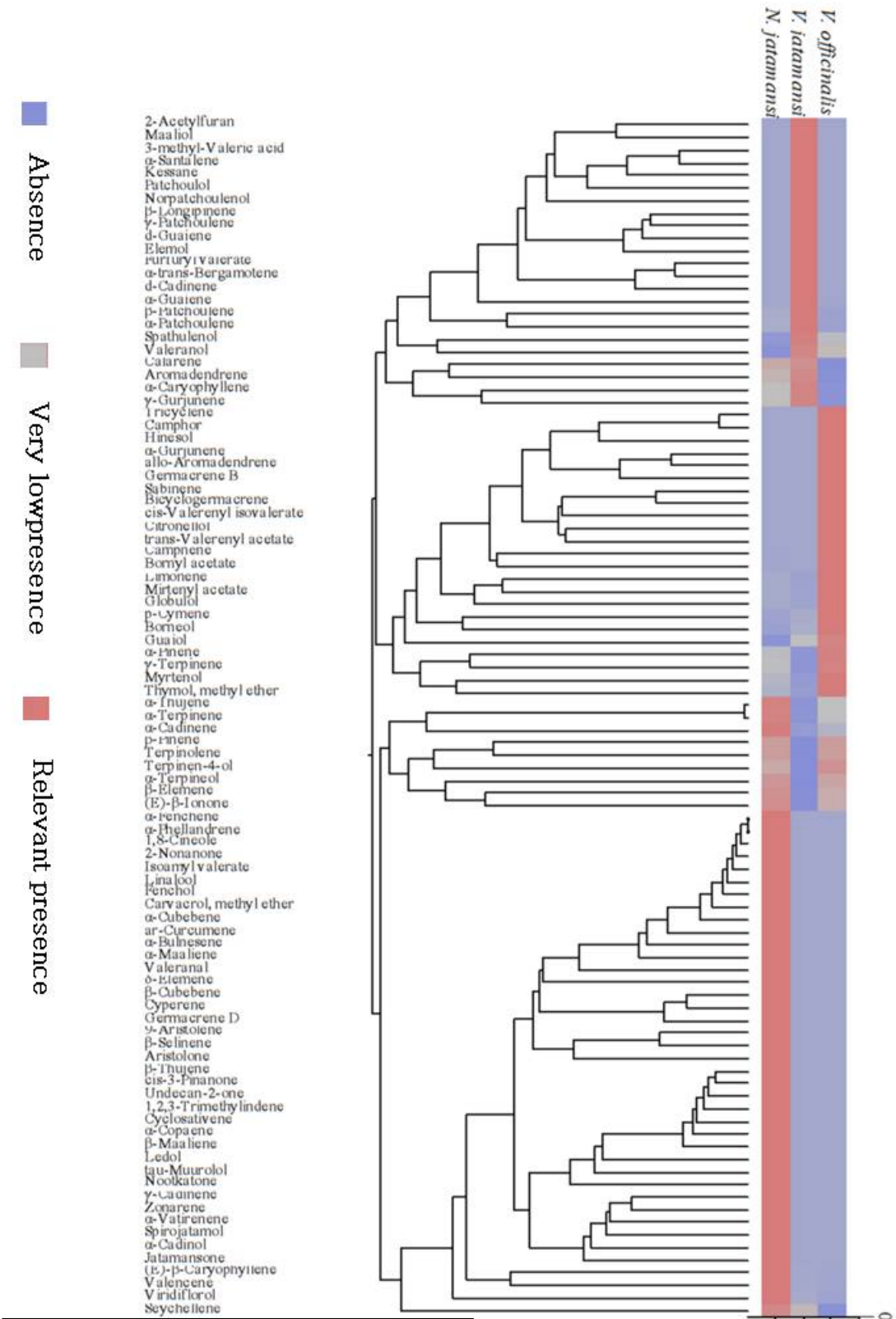

Figure 6. Dendrogram showing two clusters: one comprising Vo and Vj and another one for Nj. The color map made it possible to identify where a specific constituent was mainly represented. Blue color = complete absence of the component in the corresponding species; grey color = very low presence; red color = relevant presence of a particular compound (Smeriglio, IECPS 2020).

2.4. Acetylcholinesterase Inhibitory Activity

The effects of Nj, Vj and Vo EOs on acetylcholinesterase (AChE) activity were evaluated and compared with those of galantamine (positive control at a concentration of 7 µg/mL). Nj showed the strongest AChE-inhibitory activity, followed by Vo and Vj (Figure 7).

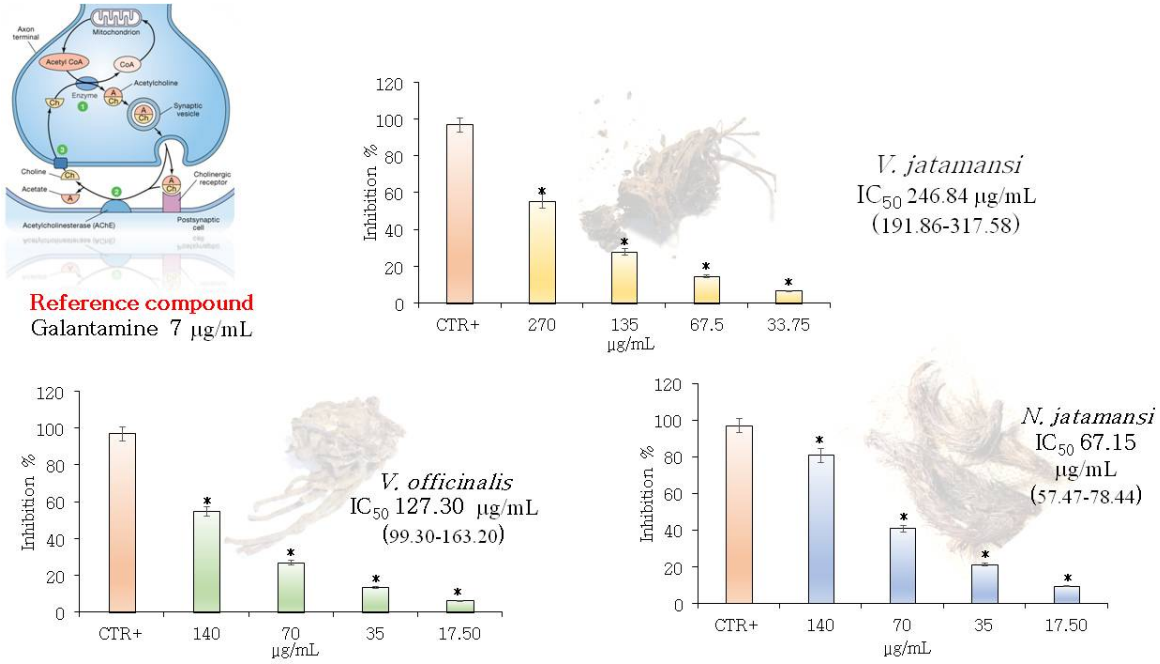

Figure 7. The AChE inhibitory activity of three Caprifoliaceae species is expressed by half-maximal inhibitory concentrations (IC_{50}) with respective confident limits (C.L.) calculated at 95% and compared with galantamine as positive control (Smeriglio, IECPS 2020).

2.5. Neuroactive Effects

The MEA technique was used to investigate changes in the spontaneous electrical activity of the cortical neuronal networks of *in vitro* mice in response to Nj, Vj and Vo EOs. The data analysis described changes in overall neuronal activity state in terms of mean firing rate (MFR) and bursting behaviour in terms of mean burst rate (MBR). The EO concentrations were administrated in cumulative concentrations on the MEA chip to obtain the concentration-response curves for MFR and MBR parameters, as shown in Figure 8.

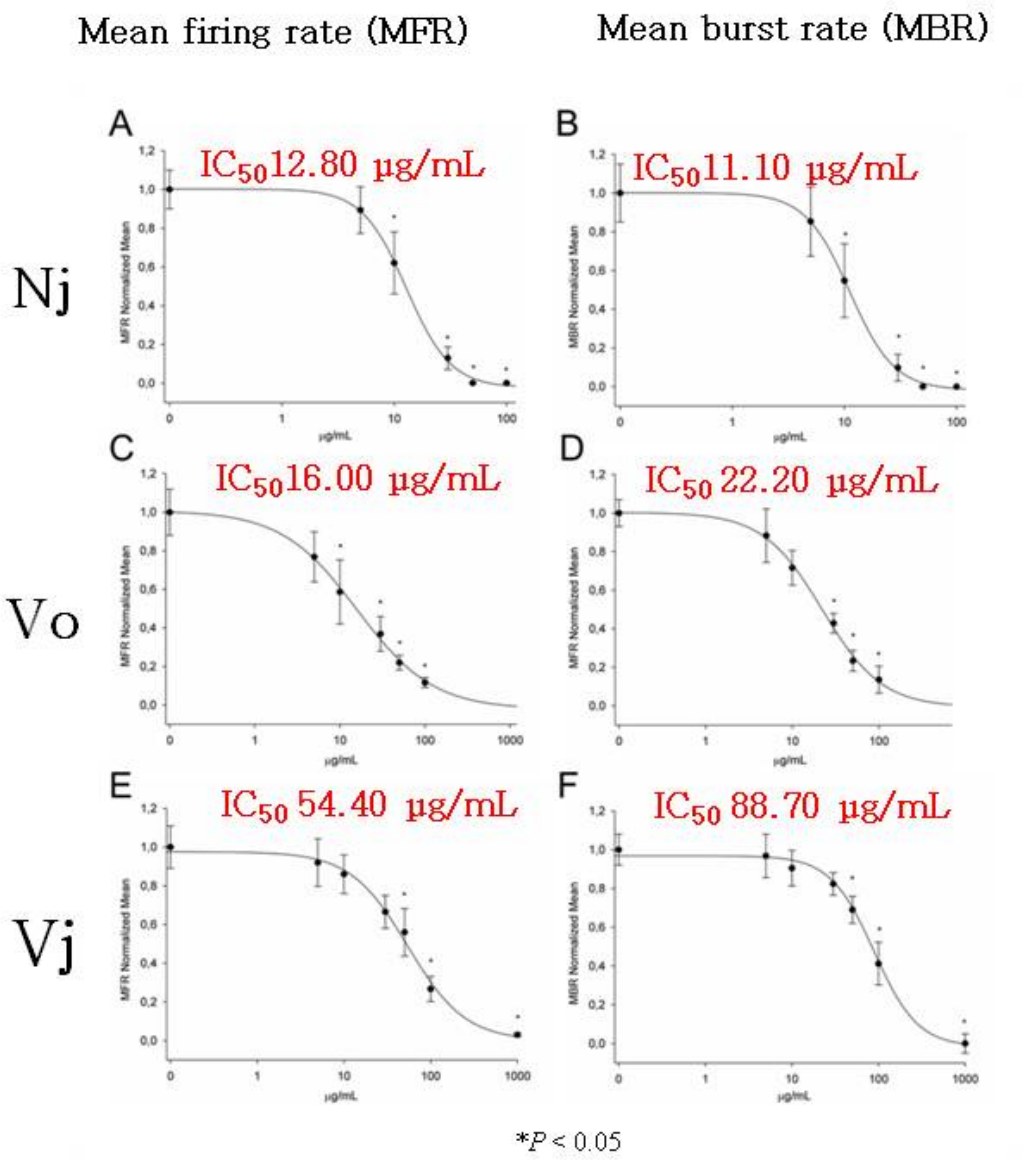

Figure 8. Neuroactive effects of Vj, Nj and Vo EOs in mice cortical neuronal networks grown on MEA microchip, expressed by half maximal inhibitory concentrations (IC_{50}) value and compared with the non-selective GABA agonist muscimol (Novellino et al., 2011) (Smeriglio, IECPS 2020).

Results showed that Nj and Vo EOs were the most potent in the inhibition of spontaneous activity, while the EO of Vj induced a decrease in neuronal activity at higher concentrations, as observed by the IC_{50} of 54.4 and 88.7 µg/mL for MFR and MBR respectively.

Plate 34. The appereance of *Nardostachys jatamansi* rhizome resembles the hairstyle of Shiva and ascetics.

Chapter 3: Discussion

Nardostachys jatamansi (D.Don) DC. (Nj) is a rare and endangered Himalayan species, from whose rhizomes are obtained important herbal remedies which are traditionally used to treat many human diseases. In this PhD research, it was possible to directly observe how traditional Nepalese healers use it for the treatment of nervous system disorders. Due to its therapeutic efficacy, *N. jatamansi* is a highly commercialised species, but because of its high price, it is frequently adulterated with other species such as the related species of Caprifoliaceae *Valeriana jatamansi* Jones ex Roxb. (Vj) and *Valeriana officinalis* L. (Vo). These two valerians have in fact been used since ancient times for their antidepressant properties (Kulkarni et al., 2010; Singh et al., 2011).

Many studies have been published on *N. jatamansi*, but there are still many aspects to be explored, such as isolation and identification of the components having therapeutic properties, and elucidation of the mechanism underlying pharmacological activities (Ahmad et al., 2013). Therefore, an interdisciplinary approach was chosen in this study to compare the three plant species of Nj, Vj and Vo.

3.1.Botanical standardisation

The first step of the study was botanical standardisation of the three Caprifoliaceae medicinal species via macroscopic and microscopic analyses of sectioned root and rhizomes as well as powdered materials. Since dried roots and rhizomes represent a recalcitrant material for this kind of study, especially for Nj, optical microscope observation was combined with SEM analysis to obtain better characterisation of these portions.

Comparing the three rhizomes, the first most significant feature was the macroscopic appearance of the Nj rhizome, which was always densely covered with the silky reddish fibrous remains of the petiole of the basal leaves. This outward appearance is probably the origin of the specific name “jatamansi” and hasspiritual value. According to the Sanskrit-English Dictionary (Williams, 1872), the word *jatamansi* is composed of “jata”, which refers to twisted hair, sometimes rolled up on the head, and “mansi”, which means “meat”, probably referring to the fibrousness of these rhizomes. The reference to hair is probably linked to iconography of the Hindu god Shiva and to the habit of Himalayan ascetics and shamans of styling their hair in a similar way.

From the microscopic point of view, the Nj rhizome has peculiar characteristics such as a pith which in a transversal section appears star-shaped and turns necrotic in the older portions,

as well as starch granules, generally occurring as simple or compound granules with few components.

All the samples were identified and confirmed successfully via DNA barcoding, indicating that DNA barcoding is a powerful tool for species identification, helping to prevent fraud and substitutions that are common for the rhizomes and roots of both valerians and Nj.

3.2.Phytochemical characterisation

The next step in characterising the three Caprifoliaceae was to define the EOs' phytochemical profiles, in order to perform further analysis on their biological activity on the nervous system. Ninety-five compounds were found, some of which were identified for the first time, while others had been reported in previous studies on plants coming from different locations and regions (Table 13).

Table 13. Comparison between phytochemicals identified in previous studies and those found in present study by analyzing EOs of Nj, Vj and Vo.

	Compounds identified in previous studies	**Compounds in common with those found in ourstudy**	**Reference**
Nardostachys jatamansi	9	6	Maiwulanjiang et al., 2015
	40	26	Costa et al., 2007
	41	19	Chauhan et al., 2017
Valeriana jatamansi	39	22	Verma et al., 2011
	20	15	Bhatt et al., 2012
	21	17	Raina et al., 2015
	23	12	Singh et al., 2015
Valeriana officinalis	53	25	Safaralie et al., 2008
	21	11	Samaneh et al., 2010
	17	7	Wang et al., 2010

The results of this comparison revealed high variability in the expression of the chemical composition of EOs, particularly for the most abundant compounds, depending on ecotype, climatic conditions and environmental interactions. For instance, Indian Vj is characterised by high amounts of patchoulol, but this metabolite is poorly expressed in our sample from Nepal compared to previous studies (Costa et al., 2007).

3.3.Biological activities assays

3.3.1. AChE inhibitory activity assay

Cholinergic disturbance is often the basis of neuropsychological impairments typical of several neurological disorders, such as Alzheimer's disease. Several aromatic plants have been used worldwide to alleviate and cure neuronal ailments. EOs and their isolated bioactive compounds could represent new therapeutic approaches (Smeriglio et al., 2018; Smeriglio et al., 2019).

Several authors have pointed out that some extracts, EOs and isolated compounds from the genus of *Valeriana* and *Nardostachys* have significant AChE inhibitory activity (Chen et al., 2016; Dong et al., 2015; Mathew & Subramanian, 2014; Mukherjee et al., 2007; Wang et al., 2010).

In this PhD research, the AChE-inhibitory activity of Vo, Vj and Nj EOs were evaluated and compared for the first time. From comparison with previous studies carried out on each species individually, it is possible to postulate that isolated compounds, mostly oxygenated and belonging to the class of sesquiterpenes, show weak inhibitory activity on AChE. It is the plant complex, extract or EO that exhibits the greatest inhibitory activity on this enzyme. This is probably attributable to the synergistic action of not only the most representative compounds of the plant complex, but also minor compounds which are often specific to each species considered.

3.3.2. MEA analysis

Previous functional screening of traditional herbal antidepressants has been performed on primary cortical neuronal networks grown on multielectrode neurochips. Gramowski et al. (2006) reported that multiparametric assessment of electrical activity changes caused by a mixture containing Vo and other psychoactive herbal extracts revealed receptor-specific and concentration-dependent inhibition of the firing patterns. Moreover, they provided evidence that the herbal extracts acted on GABA and serotonin (5-HT) receptors, which are recognised targets of pharmacological antidepressant treatments.

These results are in line with those found in this study showing concentration-dependent inhibition of spontaneous electrical activity for Vo and Nj EOs. In particular, similar concentration-response curves for MFR and MBR were observed, suggesting that they have a similar mode of action. However, the dissimilar chemical profile of Nj and Vo did not allow us to speculate about any compound potentially responsible for the significant effect on electrical activity induced by the two EOs. For this purpose, further experiments will have to

be carried out using the most abundant pure compounds present in the EOs in order to detect which ones are responsible for inhibiting the neuronal activity. The Vj EO was the least effective in inhibiting spontaneous electrical activity on neuronal cultures. In addition, in this case, the two derived activity parameters were affected with different potencies. This strongly suggests that Vj EO acts through different targets that affect the number of spikes in a burst.

Chapter 4: Conclusion

At the end of this second part of PhD project, some conclusions can be drawn:

- Microscopic and DNA barcoding analysis represents a rapid and valid approach to herbal drug identification, allowing discrimination between genera and species.
- These techniques, combined with phytochemical fingerprinting of EOs, are important tools to avoid adulteration of herbal drugs and to discriminate between the EOs of plants coming from different locations.
- This study demonstrates experimentally, for the first time, the effects on the central nervous system of the EOs of three Caprifoliaceae species traditionally used for their anxiolytic properties: *N. jatamansi*, *V. jatamansi* and *V. officinalis.* Validation of such properties and comparison of the effects of the three EOs was achieved both via an AChE inhibitory activity test and MEA array on a reconstituted murine neuronal network *in vitro*.
- This model reduces the number of animals used to a minimum, in accordance with the principles of the 3Rs (Replacement, Reduction and Refinement), and represents an absolute innovation in the pharmacological/toxicological field for investigating the effects of plant complexes on the central nervous system.
- As such, our methods can be recommended for correct identification of herbal drugs and evaluation of the effectiveness of EOs used in the therapeutic treatment of nervous disorders.

General conclusions

The ethnobotanical information collected from people living in the rural areas of Kavrepalanchok District (Central Nepal) showed richness of cultural traditions and knowledge about uses of plants for the needs of daily life and for treating the most common ailments. This heritage has been transmitted orally for ages, but today this traditional knowledge is in danger of being lost due to many economic and social factors. Avoiding this risk requires not only correct documentation, but also scientific validation, in order to preserve and promote the traditional usage of medicinal plants.

First, I carried out an ethnobotanic investigation in the field, which made it possible to collect a great deal of information about the local use of plants. Then, I focused my attention on certain species with sedative and anxiolytic properties that are widely used for medicinal purposes by shamans. These plants were the subject of an in-depth multidisciplinary study.
Such a multidisciplinary approach — including microscopic, genetic and phytochemical characterisation, as well as biological assays (AchE test and MEA arrays) — represents an important approach for future screening of plant bioactive compounds.

The set of data obtained could lead to the development of medicinal plant cultivations in the dismissed agricultural lands of Central Nepal, turning them into a source of income for local people.

A recent example in the Temal rural area of Kavrepalanchok District concerns an endemic species, *Ziziphus buddhensis* Bhattarai & M.L. Pathak. This tree has great spiritual and economic value. Indeed, it is cultivated for its woody seeds, sold to make beads for Buddhist rosaries, bracelets, necklaces, and lockets (Lama et al., 2019). However, the pericarp of its fruits, despite being edible, is often discarded and unused. It could be subjected to pharmacognostic investigation, which in turn could encourage local people to be actively involved in future projects aimed at sustainable use of natural resources.
During the field study, we recorded that there is great demand for *Asparagus racemosus* Willd. roots in the study area because they are useful for stimulating milk production in buffaloes. For this reason, the collection of, and trade in, this species should be controlled to prevent environmental depletion, while the sustainable cultivation of this species should be enhanced.

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Dr. Ambu Gabriele

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APPENDIX I

The following articles have already been published in Gold Open Access at the MDPI Publisher, with the following licenses CC-BY:

Ambu, G.; Chaudhary, R.P.; Mariotti, M.; Cornara, L. Traditional Uses of Medicinal Plants by Ethnic People in the Kavrepalanchok District, Central Nepal. Plants 2020, 9, 759. https://doi.org/10.3390/plants9060759.

Article

Traditional Uses of Medicinal Plants by Ethnic People in the Kavrepalanchok District, Central Nepal

Gabriele Ambu [1], Ram Prasad Chaudhary [2], Mauro Mariotti [1] and Laura Cornara [1,*]

1 Department of Earth, Environment and Life Sciences, University of Genova, 16132 Genova, Italy; frategabriele@libero.it (G.A.); m.mariotti@unige.it (M.M.)
2 Research Centre for Applied Science and Technology (RECAST), Tribhuvan University, 44613 Kathmandu, Nepal; ram.chaudhary53@gmail.com
* Correspondence: laura.cornara@unige.it or cornaral@gmail.com; Tel.: +39-010-209-9364

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Abstract: In rural areas of Nepal, where it is difficult to get access to Government health care facilities, people depend on medicinal plants and local healers for health problems. This study concerns an ethnobotanical survey of the Kavrepalanchok District, reporting some unusual uses of medicinal plants and original recipes. A total of 32 informants were interviewed, 24 of them being key informants. Ethnobotanical uses concerned 116 taxa, of which 101 were medicinal plants, with the most representative species belonging to Asteraceae, Fabaceae, Lamiaceae, and Zingiberaceae. Ethnobotanical indexes were used to evaluate the ethnopharmacological importance of each plant species and the degree of agreement among the informants' knowledge. Informant consensus factor (Fic) showed that the fever category had the greatest agreement. Highest fidelity level (FL) values were found for *Calotropis gigantea* used for dermatological diseases, *Drymaria cordata* for fever, *Mangifera indica* and *Wrightia arborea* for gastrointestinal disorders. Data document the richness of the local flora and the traditional knowledge on medicinal plant species used by ethnic communities in rural areas. The active involvement of local populations in the conservation and management of medicinal plant species will encourage future projects for the sustainable development of the biological and cultural diversity of these rural areas of Nepal.

Keywords: traditional ecological knowledge; ethnopharmacology; medicinal plants; conservation

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Article

Comparative and Functional Screening of Three Species Traditionally used as Antidepressants: *Valeriana officinalis* L., *Valeriana jatamansi* Jones ex Roxb. and *Nardostachys jatamansi* (D.Don) DC.

Laura Cornara [1], Gabriele Ambu [1], Domenico Trombetta [2], Marcella Denaro [2], Susanna Alloisio [3,4], Jessica Frigerio [5], Massimo Labra [6], Govinda Ghimire [7], Marco Valussi [8] and Antonella Smeriglio [2,*]

1 Department of Earth, Environment and Life Sciences, University of Genova, 16132 Genova, Italy; laura.cornara@unige.it (L.C.); frategabriele@libero.it (G.A.)
2 Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Via Giovanni Palatucci, 98168 Messina, Italy; dtrombetta@unime.it (D.T.); mdenaro@unime.it (M.D.)
3 ETT Spa, via Sestri 37, 16154 Genova, Italy; susanna.alloisio@ettsolutions.com
4 Institute of Biophysics-CNR, 16149 Genova, Italy
5 FEM2 Ambiente Srl, Piazza della Scienza 2, 20126 Milan, Italy; jessica.frigerio@fem2ambiente.com
6 Department of Biotechnology and Bioscience, University of Milano-Bicocca, Piazza della Scienza 2, 20126 Milan, Italy; massimo.labra@unimib.it
7 Nepal Herbs and Herbal Products Association, Kathmandu 44600, Nepal; ghimiregovinda31@gmail.com
8 European Herbal and Traditional Medicine Practitioners Association (EHTPA), Norwich 13815, UK; marco@gadoi.it
* Correspondence: asmeriglio@unime.it; Tel.: +39-0906-764-039

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Abstract: The essential oils (EOs) of three Caprifoliaceae species, the Eurasiatic *Valeriana officinalis* (Vo), the Himalayan *Valeriana jatamansi* (Vj) and *Nardostachys jatamansi* (Nj), are traditionally used to treat neurological disorders. Roots/rhizomes micromorphology, DNA barcoding and EOs phytochemical characterization were carried out, while biological effects on the nervous system were assessed by acetylcholinesterase (AChE) inhibitory activity and microelectrode arrays (MEA). Nj showed the highest inhibitory activity on AChE (IC_{50} 67.15 µg/mL) followed by Vo (IC_{50} 127.30 µg/mL) and Vj (IC_{50} 246.84 µg/mL). MEA analyses on rat cortical neurons, carried out by recording mean firing rate (MFR) and mean bursting rate (MBR), revealed stronger inhibition by Nj (IC_{50} 18.8 and 11.1 µg/mL) and Vo (16.5 and 22.5 µg/mL), compared with Vj (68.5 and 89.3 µg/mL). These results could be related to different EO compositions, since sesquiterpenes and monoterpenes significantly contribute to the observed effects, but the presence of oxygenated compounds such as aldehydes and ketones is a discriminating factor in determining the order of potency. Our multidisciplinary approach represents an important tool to avoid the adulteration of herbal drugs and permits the evaluation of the effectiveness of EOs that could be used for a wide range of therapeutic applications.

Keywords: Caprifoliaceae; essential oil; acetylcholinesterase; neuroactive effects; MEA analyses; DNA barcoding; micromorphology; botanicals authentication

APPENDIX II

Ambu G., Lama B., Cornara L. 2017. Uomini, piante e tradizioni del Nepal. In cammino tra i villaggi dei Tamang. Erboristeria Domani, 407, 80-87. **ISSN 1721-5676.**

Abstract: *The population of Nepal, severely hit by a violent earthquake in 2015, is made up of a multitude of groups and ethnic groups whose traditional knowledge risk being compromised by the difficult living conditions. The article reports on the ethnobotanical customs and traditions of the Tamang people living in the hilly areas around Kathmandu.*

Ambu G., Cornara L. 2019. Nardo, profumo di spiritualità. Erboristeria Domani, 415, 84-92. **ISSN 1127-6320.**

Abstract: *Valeriana jatamansi and Nardostachys jatamansi are two Caprofoliaceae used by Nepalese shamans for their healthy properties. The article deal with these species, generally known like "spikenard", their botanical characteristics, utilization history in traditional medicine, and folklore.*

APPENDIX III

Table reporting plants and their traditional uses by ethnic people in Kavrepalanchok District, Central Nepal (Ambu et al., 2020).

Table 2. Plants used by ethnic people in Kavrepalanchok District, Central Nepal.

Family Scientific Name Voucher Number	Vernacular Name [a]	Origin [b]	Parts Used [c]	Ethnobotanical Uses [d]	Uses Description	Similar Uses References	Other Uses References
Acanthaceae							
Justicia adhatoda L. GA20171031NP011	Asuro (N) Basak (T)	W	L R	agr med/resp,fev,urogen med/fev	The leaves are used in horticulture as fertilizer. Dried leaves are smoked to cure cough. Powder of 4 dried leaves is mixed with honey (or water) and eaten, twice a day (morning and evening), to treat fever. In case of urinary problems: 3 dry leaves, after being left in water for 5 h, are cut into small pieces, honey is added, and the final product is eaten. Root juice is filtered and drunk 3 times a day to treat high fever.	[23,24,26,27]	Typhoid [23], rheumatic pain [24], sinusitis [25]
Strobilanthes pentastemonoides (Nees) T. Anderson (= *Goldfussia pentastemonoides* Nees) GA20181012NP052	Gathe (N)	W	R	med/fev	The root is cut into small pieces, pounded with water and the filtered juice is drunk 3 times a day to cure high fever.		
Amaranthaceae							
Achyranthes bidentata Blume GA20171113NP047	Dok, Datiwan (N), Ghyurupuchu, Ghiughiuru (T)	W	R Wp	med/fev med/mat med/urogen vet med/met,fev,derm	Root is crushed with water and the filtrate is drunk 3 times per a to cure fever and typhoid. One day of treatment is often sufficient for healing. Root juice is drunk 2 times day to promote childbirth. The juice obtained by squeezing the root is drunk to treat urinary problems (blood in the urine). Root juice stimulates lactation in buffaloes. Plant juice is drunk for blood purification and to cure fever. The well-washed plant is rubbed directly on the skin, to treat pimples, boils, itchy pustules.	[16]	Asthma [16], gastric problems, toothache [17]
Amaranthus cruentus L. GA20171030NP002	Latte (N)	C	L/Sd	food	The leaves are cooked like spinach, added to soups, or eaten raw. The seeds are cooked and used for soups (*satoo*), or ground to obtain a flour for bread (*chapati*).		
Amaranthus hybridus L. GA20171030NP003	Latte (N)	C	L/Sd	food	The leaves are cooked like spinach, added to soups, or eaten raw. The seeds are cooked and used for soups (*satoo*), or ground to obtain a flour for bread (*chapati*).		

Table 2. *Cont.*

Family Scientific Name Voucher Number	Vernacular Name [a]	Origin [b]	Parts Used [c]	Ethnobotanical Uses [d]	Uses Description	Similar Uses References	Other Uses References
Amaranthus viridis L. GA20181016NP098	Gaute bangan (T)	W	R	med/fev	Root juice is used to cure high fever.		
Anacardiaceae							
Choerospondias axillaris (Roxb.) B. L. Burtt & A. W. Hill GA20171113NP048	Lapsi (N)	C	Fr	food	Fruits are eaten fresh or pickled.		
Mangifera indica L. GA20181017NP104	Aanp (N)	C	Fr	med/gast	The peel of fruits is boiled and eaten to cure gastritis.	[16,23,25]	Jaundice,rheumatism [16],dysentery [23]
Searsia parviflora (Roxb.) F. A. Barkley (= *Rhus parviflora* Roxb.) GA20181016NP093	Satibro (T)	W	Fr	med/gast	Ripe fruits are eaten to treat stomach problems and diarrhea.		
Apiaceae							
Centella asiatica (L.) Urb. GA20181018NP111	Tajoimra (T), Kholachagaian, Kolacha (Ne)	W	Wp R	med/fev,ant,musc,nerv med/urogen	Plant juice is drunk to cure fever and sometimes is mixed with the juice of *Drymaria cordata* to treat high fever. A typical recipe (*) is used to cure fever, internal fever, food poisoning, joint pain, migraine. Root juice is drunk to cure urinary problems.	[19,23,26,27]	Blood purification [19], indigestion [20], skin diseases [24], sinusitis [25]
Apocynaceae							
Calotropis gigantea (L.) Dryand. GA20171030NP004	Arka (sanskrit), Aank, Akh (T)	W	Lx	med/derm,musc	Latex is used directly on the skin to stop bleeding wounds and to treat ankle sprains.		Joint fracture [23]
Catharanthus roseus (L.) G. Don GA20171101NP017	Barhamase phul (N)	N	Wp	dom	The plant is used as an ornamental in home gardens.		
Wrightia arborea (Dennst.) Mabb. GA20181017NP108	Glemindhu (T)	W	Fr/Sd	med/gast,fev	The soft septum between one seed and the other, is eaten to cure flatulence, intestinal pain with dark stools, insolation with fever.		
Araceae							
Acorus calamus L. GA20181016NP096	Seda (T)	C	Wp	med/derm	The plant is grated on a stone and applied on skin wounds.		Cough/cold [19], roundworm, hookworm [23], fever [26]
Colocasia esculenta (L.) Schott GA20181015NP087	Pidalu (N), Taia (T)	C	Rh	med/gast food	Some pieces of boiled rhizome are eaten to cure the constipation of children (1–5 years). Boiled rhizome is commonly eaten as food by the people, especially for breakfast.		

Table 2. *Cont.*

Family Scientific Name Voucher Number	Vernacular Name [a]	Origin [b]	Parts Used [c]	Ethnobotanical Uses [d]	Uses Description	Similar Uses References	Other Uses References
Rhaphidophora glauca (Wall.) Schott GA20171111NP045	Birlahra (T)	W	Wp	med/mat	To promote pregnancy, the juice of the plant is taken 3 times a week: Saturday,Tuesday,Thursday.		
Asparagaceae							
Agave cantala (Haw.) Roxb. ex Salm-Dyck GA20171030NP001	Ketuki (N)	N	L	handicr	Dried leaves are used for roofing. Leaves provide fibers used to make ropes, cordage, and twine.		Diuretic, anti-syphilitic [20], worms on wound [27]
Asparagus racemosus Willd. GA20181015NP084	Kurilo (N)	C	R	med/met vet	Root juice is drunk to cure jaundice. Root juice is mixed with corn flour and given to the buffaloes for about 2–3 days, twice a day, to stimulate milk production.	[19,23]	Urinary disorders, stimulation of milk production in women [19]
Asteraceae							
Ageratina adenophora (Spreng.) R. M. King & H. Rob. GA20171109NP023	Banmara (N)	W	L	med/derm	Five to seven leaves are crushed with water and the green liquid extracted from the obtained paste is applied on cuts and wounds to stop the bleeding.	[16,17,19,25–27]	Fever, eyes insomnia [16]
Artemisia indica Willd. GA20171109NP025	Titepati (N), Dusun (Ne)	W	Wp Ys	rel med/ant,resp,fev,musc,nerv med/resp	The dried plant is used as incense. Plant juice is drunk to treat food poisoning and cough. A typical recipe (*) is used to cure fever, internal fever, food poisoning, joint pain, migraine. 4–5 shoots are crushed with water, the juice is filtered and drunk for the treatment of throat irritations.	[17]	Gastritis, paralysis, [23], rheumatic pain [24], cuts, scabies [25], louse, worms [27]
Bidens pilosa L. GA20181018NP110	Buk tinai (T)	N	Fl	med/fev,nerv	The juice of flowers is drunk, twice a day (morning and evening), to cure fever and migraine.		Cuts and wounds [16]
Blainvillea acmella (L.) Philipson (= *Spilanthes acmella* (L.) L.) GA20181012NP054	Saprumo (T)	N	R	med/resp	Root juice is drunk twice a day (morning and evening) to cure cough and cold.		
Blumea aromatica DC. GA20181015NP085	-	N	Fl	food	The dried flowers are mixed with *Clematis buchananiana* and *Oryza sativa*, and fermented for a week to obtain a popular alcoholic drink (*chhaang*).		
Cirsium wallichii DC. GA20181012NP061	Thakal (N), Achangpolo (T), Chwacan (Ne)	W	R	med/urogen,genh,fev	Root juice is drunk to treat urinary problems (blood in the urine), weakness (the juice is taken twice a day), malarial fever.		

Table 2. *Cont.*

Family Scientific Name Voucher Number	Vernacular Name [a]	Origin [b]	Parts Used [c]	Ethnobotanical Uses [d]	Uses Description	Similar Uses References	Other Uses References
Duhaldea cappa (Buch.-Ham. ex D. Don) Pruski & Anderb. (= *Inula cappa* (Buch. Ham. ex D. Don) DC.) GA20181013NP069	Ranaven, Ranabhyang (T)	W	R	med/fev	Root juice is drunk to cure fever.		Snake bite, menstrual disorders, epilepsy [19], headache [23], gastritis, indigestion [24]
Eclipta prostrata (L.) L. GA20181013NP066	Mashi mra (T)	W	St/L	med/derm	The hand-crushed plant is applied directly to the wounds of the skin.		
Elephantopus scaber L. GA20181013NP067	Buti jhar (N), Tinai (T)	W	R Wp	med/ant med/met	Root juice is drunk once/twice times in the nighttime, in case of food poisoning. Plant juice is taken to cure jaundice. In case of infant jaundice, the mother drinks the juice before breastfeeding the sick child.		Headache, sinusitis [23]
Galinsoga parviflora Cav. GA20171113NP049	Bhuitimur (N)	N	Fl	med/ENT	3 flowers are put on the sore tooth for 15–30 min. Once is often enough to relieve toothache, but the treatment can be repeated a second time after 3 days.		
Smallanthus sonchifolius (Poepp.) H. Rob. GA20171113NP051	Bhuishyau (N)	C	R	med/met food	The tuberous root, cleaned and eaten raw, once per week, is useful in case of diabetes. Tubers are slightly sweet, crispy and juicy and are usually eaten raw like a fruit.		
Tagetes erecta L. (= *Tagetes patula* L.) GA20171030NP005	Sayapatri (N), Saipatri sun (Ne)	C	Fl	rel med/derm,fev,ant,musc,nerv	Flowers are used for the creation of garlands and decorations for weddings, festivals, and other religious events. Flowers are rubbed on the injured and swollen skin. A typical recipe (*) is used to cure fever, internal fever, food poisoning, joint pain, migraine.		
Berberidaceae							
Berberis asiatica Roxb. ex DC. GA20181012NP059	Chutro (N)	W	R	med/fev,ENT,gast	The root is boiled for 5 minutes in about 1/2 L of water and the infusion is drunk to treat fever, toothache, "red eyes" (conjunctivitis), mouth infections, diarrhea.	[19,20,25]	Blood purification [19]
Bignoniaceae							
Oroxylum indicum (L.) Kurz GA20181018NP113	Tatelo (N), Tarlason (Ne)	W	Sd	rel med/derm fev,ant,musc,nerv	Seeds are widely used in religious ceremonies and divinatory practices of shamans. Seed are applied externally to heal cuts and wounds. A typical recipe (*) is used to cure fever, internal fever, food poisoning, joint pain, migraine.	[17,26,27]	Dysentery [26], jaundice [27]

Table 2. *Cont.*

Family Scientific Name Voucher Number	Vernacular Name [a]	Origin [b]	Parts Used [c]	Ethnobotanical Uses [d]	Uses Description	Similar Uses References	Other Uses References
Brassicaceae							
Brassica rapa L. (= *Brassica campestris* var. *sarson* Prain) GA20171031NP006	Sarson (N)	C	Sd	food med/ENT med/musc	The oil obtained by seeds is used to cook. The ointment obtained mixing hot rapeseed oil and dried bark of *Myrica esculenta*, is applied externally to treat earache. The ointment obtained mixing hot rapeseed oil with leaves of *Abrus precatorius*, is used for massages in case of joint pain, twice a day.	[16]	
Capparaceae							
Crateva religiosa G. Forst. GA20181015NP088	Siplekan (N)	W	Fr	med/derm,ENT	Dried fruits are grated on a stone with the addition of water and the mixture is applied to the wounds of the skin. The fresh fruit liquid is applied externally to treat the swelling of the dental glands and the affected part is bandaged with "Nepali paper".		
			L	med/gast	The young leaves are boiled and eaten to treat stomach pain.		
Caprifoliaceae							
Valeriana hardwickii Wall. GA20171112NP046	Daling (T)	W	R	med/gast	Fresh root juice or infusion of the dried root, is taken twice a day for a week, to cure gastric problems and vomiting.		
Valeriana jatamansi Jones ex Roxb GA20181015NP082	Jatamasi (N), Dhalin (T)	W	R	med/gast,nerv	Root juice is taken to treat diarrhea in children. With the dried roots shamans produce the incense for the treatment of anxiety and insomnia.		Fire burns [26]
				rel	Incense from the plant root is used for religious purposes.		
Caricaceae							
Carica papaya L. GA20171031NP007	Papaya (I)	C	Fr	food	Plant is cultivated in kitchen gardens for its edible fruits.		
Caryophillaceae							
Arenaria benthamii Fenzl ex Torr. and A. Gray GA20181012NP057	Tangne (T)	W	Wp	med/resp	The pillows are filled with the plant so that the active ingredients are inhaled during the night to cure fever and breathing problems.		

Table 2. *Cont.*

Family Scientific Name Voucher Number	Vernacular Name [a]	Origin [b]	Parts Used [c]	Ethnobotanical Uses [d]	Uses Description	Similar Uses References	Other Uses References
Drymaria cordata (L.) Willd. ex Schult. GA20171111NP042	Abijalo (N),Tangar, Abijal (T), Abisal ghe, Kai bugain (Ne)	W	Wp	med/fev,gast,ant,resp,musc,nerv	The plant parts are pounded, boiled in water for about 5 min and the filtrate is drunk once a day (morning or evening) for 3 days, to cure fever. Plant juice is drunk twice a day to cure fever and stomach infections. Plant juice is drunk to treat food poisoning, rhinitis, and sinusitis. Sometimes the plant juice is mixed with that of *Centella asiatica* to cure high fever. A typical recipe (*) is used to cure fever, internal fever, food poisoning, joint pain, migraine.	[24,25]	
Combretaceae							
Terminalia bellirica (Gaertn.) Roxb. GA20181017NP107	Barla (T)	W	Fr	med/resp,gast	Fruits are kept in the mouth and sucked like candy to cure cough and gastric problems.	[20,23,26,27]	Cold, cough [27]
Convolvulaceae							
Cuscuta reflexa Roxb. GA20181013NP065	Sikari lahara (N) Sky grass (english)	W	St	med/met	Used in case of jaundice: (a) the plant juice is drunk; (b) the plant is pounded, boiled in water for 2–3 min and the filtrate is drunk.	[19,20,23–27]	Bone fractures, body swelling [19], roundworm, depression [23]
Coriaraceae							
Coriaria nepalensis Wall. GA20181017NP101	Bhujinshin (N) Hakupaku (T)	W	Fr L/St	med/gast	Ripe fruits and plant juice are taken to treat indigestion.		
Crassulaceae							
Bryophyllum pinnatum (Lam.) Oken GA20181012NP060	Kidney stone medicine	N	L	med/urogen	Leaf juice is drunk, or the leaves are eaten raw to cure urinary problems.		
Cucurbitaceae							
Momordica charantia L. GA20181016NP090	Karela (N)	C	Fr	med/card food	Fruits are consumed to control high blood pressure. Unripe fruits are cooked as a vegetable or pickled.		
Dioscoreaceae							
Dioscorea bulbifera L. GA20171110NP032	Dhingyui mindhu (T)	W	R	med/mat	The root (white color) is cut into small pieces and crushed to obtain a red juice, taken once a day regularly, by women with menstrual cycle disorders, for preventive purposes.		Piles, dysentery, syphilis, ulcers [16], pneumonia [19]

Table 2. *Cont.*

Family Scientific Name Voucher Number	Vernacular Name [a]	Origin [b]	Parts Used [c]	Ethnobotanical Uses [d]	Uses Description	Similar Uses References	Other Uses References
Euphorbiaceae							
Euphorbia hirta L. GA20181017NP103	Rato lahare (N) Walagughi (T)	W	Lx	med/derm,musc	Latex is applied on skin wounds and joint trauma without bone fractures.	[16,26]	Diarrhoea/dysentery, respiratory diseases, snake bites [16], excessive menstrual flow [24]
Jatropha curcas L. GA20171031NP010	Arin,Sajiwan (N) Mandhar (T)	N	St Fr/Lx	med/ENT med/derm	The most tender twigs are used for cleaning the teeth. Dried fruits or latex are applied externally in case of skin infections.	[23,24,26]	Gum problems [24]
Ricinus communis L. GA20181018NP116	Taturoro (Ne)	N	Fl	vet	The flowers are pounded with water and the paste obtained is applied to treat skin problems of cattle.		Bone fractures [23], worms in the teeth [24]
Fabaceae							
Abrus precatorius L. GA20181012NP055	Rati geri (N)	W	R L	med/nerv,resp,ant med/musc	The root is grated on a stone and inhaled to treat migraine. The root is grated on a stone, mixed with honey, and taken to cure cough. The dried root is pulverized and applied on snake bites, and the affected part is banded with "Nepali paper". The ointment obtained by cooking the leaves with rapeseed oil is used to massage the aching joints, twice a day.		Stomach problems [27]
Albizia julibrissin Durazz. GA20181016NP097	Shirish (N)	W	R	med/musc	The root is cut into small pieces and boiled for more than 3 h along with *Osyris wightiana* and *Senegalia catechu*; the filtered juice is applied externally in case of bone fractures and the affected part is bandaged with "Nepali paper".		
Bauhinia variegata L. GA20181012NP058	Koiralo (N)	W	Br	med/ENT,met,ant	The bark is boiled in about half liter of water. So water is used for gargling, in case of mouth infections and toothache. Even the dried bark can be used to treat toothache. The dried bark is powdered, mixed with *Zingiber officinale* powder and water. The dough obtained is applied externally on the throat, twice a day (morning and evening) in the treatment of goiter. Dried and pulverized bark is mixed with the juice of *Citrus* × *limon*; the dough obtained is applied on snake bites and the part is bandaged.		Diarrhoea/dysentery, piles [20], gastritis [25], fever [27]

Table 2. *Cont.*

Family Scientific Name Voucher Number	Vernacular Name [a]	Origin [b]	Parts Used [c]	Ethnobotanical Uses [d]	Uses Description	Similar Uses References	Other Uses References
Lablab purpureus (L.) Sweet GA20171110NP033	Lahare guki (T)	C	L	med/gast	The leaves are boiled for about 5–10 minutes, resulting in a *daal*-like soup, which is consumed regularly to treat *kabjiat* (constipation).		Ringworm on skin [27]
Phaseolus vulgaris L. GA20171031NP014	Rajama (N)	C	Fr/Sd	food	Fruits and seeds are used as vegetable and for cooking *daal* (lentil soup).		
Saraca asoca (Roxb.) J.J.de Wilde GA20181017NP106	Ashoka (N)	W	Sd	med/musc	The seeds are taken with water to bring relief in case of bone fractures.		
Senegalia catechu (L. f.) P.J.H.Hurter and Mabb. (=*Acacia catechu* (L. f.) Willd.) GA20181016NP095	Khayar (T)	W	R	med/musc	The root is cut into small pieces and boiled: the decoction is applied externally to promote the healing of bone fractures. Another recipe: small pieces of root are boiled for more than 3 h along with *Osyris wightiana* and *Albizia julibrissin*; the liquid obtained is applied on the affected part and the part is banded with "Nepali paper".	[26]	Diarrhoea/dysentery [23], fever [26]
Gentianaceae							
Swertia angustifolia Buch.-Ham. ex D. Don GA20181014NP079	Chiraito (N), Kampman (T)	W	R/L Wp	med/fev	To treat fever: (a) root and leaf juice is drunk; (b) the whole plant is put in water for 12 h, then the macerate is drunk twice a day.	[16]	Blood purification, bile diseases, cough/cold [16]
Iridaceae							
Iris domestica (L.) Goldblatt & Mabb. GA20171110NP029	Darware mindhu (T)	C	R	med/gast,ant	The root juice is used in case of gastric problems and poisoning, once a day, in the morning.		Diarrhoea [27]
Lamiaceae							
Colebrookea oppositifolia Sm. GA20171031NP008	Dhursil (N), Busul sul (T)	W	Fl St L	rel med/derm med/gast	Flowers are sold in urban markets for temple offerings. Thin stem filaments are applied to cuts and wounds and the affected part is bandaged. Hand-crushed leaf juice is drunk by children in case of liquid diarrhea accompanied by abdominal pains.	[16,17]	Epilepsy, fever, headache, sinusitis [16], arthritis [17]
Leucas cephalotes (Roth) Spreng. GA20181013NP070	Topdoi mra (T)	W	Fl	med/met,musc	The infusion of flowers is drunk to cure jaundice and joint pain.		
Mentha spicata L. GA20181018NP112	Naasun (Ne)	W	Fl	med/fev,ant,musc,nerv	A typical recipe (*) is used to cure fever, internal fever, food poisoning, joint pain, migraine.		Gastric and intestinal disorders [16], insomnia [25]

Table 2. *Cont.*

Family Scientific Name Voucher Number	Vernacular Name [a]	Origin [b]	Parts Used [c]	Ethnobotanical Uses [d]	Uses Description	Similar Uses References	Other Uses References
Ocimum tenuiflorum L. GA20171031NP012	Tulsi (N)	C	Wp	rel med/gast,ENT	The plant is considered sacred and is used in the worship of Vishnu. The plant is used to prepare a herbal tea useful for gastric problems and dry mouth.		Blood pressure control, ear pain, respiratory diseases, typhoid [23]
Perilla frutescens (L.) Britton GA20171031NP013	Silam (N)	W	Sd/L	food	Toasted seeds are used to prepare a spicy sauce (*silam ko achar*); the leaves cooked like spinach; dried leaves are used to prepare an healthy herbal tea.		
Pogostemon benghalensis (Burm. f.) Kuntze GA20171111NP044	Rutula (T)	W	Wp	med/resp	Plant juice is filtered and drunk, twice a day for a week, to treat dry and tickly cough.	[23,27]	Typhoid [23]
Lauraceae							
Cinnamomum glanduliferum (Wall.) Meisn. GA20181016NP099	Tagba (T)	W	R	med/musc	The root is crushed, boiled for more than an hour and the mush obtained is used to massage the painful joints, twice a day.		Toothache, wounds [19]
Litsea cubeba (Lour.) Pers. GA20181013NP071	Siltimur (N)	W	Fr	med/gast	The dried ripe fruits, pulverized and dissolved in water, are taken twice a day to treat stomach problems.		Cholera [20]
Machilus odoratissima Nees (= *Persea odoratissima* (Nees) Kosterm. GA20181014NP076	Kaulo (N)	W	Br	med/card,musc,urigen	Dried bark is cut into small pieces, reduced to powder, mixed with honey and taken 7 times a day. Useful for heart attack, bone fractures, poor blood circulation, urinary problems.		
Lythraceae							
Woodfordia fruticosa (L.) Kurz GA20171110NP038	Daduimre, Bhyur ghara (T)	W	R Ys	med/gast	Root juice is drunk for stomach problems. The juice of about 1 kg of twig young shoots (red color) is drunk once/twice times a day to treat abdominal pain with blood in the stool.	[20,23,25–27]	
Malvaceae							
Gossypium arboreum L. GA20171031NP009	Kopi (T)	C	Sd	handicr	The fibres from the seeds are used in the production of blankets and wicks for incense.		
Melastomataceae							
Osbeckia nepalensis Hook. GA20181014NP075	Chulsi (N)	W	Wp	med/derm	The mixture obtained by crushing and mixing the plant with *Rubus ellipticus* whole plant, is applied directly on skin infections that tend to expand, especially on the abdomen.	[24]	Fever [27]

Table 2. *Cont.*

Family Scientific Name Voucher Number	Vernacular Name [a]	Origin [b]	Parts Used [c]	Ethnobotanical Uses [d]	Uses Description	Similar Uses References	Other Uses References
Meliaceae							
Cipadessa baccifera (Roth) Miq. GA20181016NP100	Painati (T)	W	Br	med/ant	The juice from some stem slices is drunk to cause vomiting against the food/drink poisoning.		Cough/cold [16]
Melia azedarach L. GA20181017NP105	Bakaino, Bakena (N)	W	Br	med/nerv,resp,fev,gast	The bark powder, mixed with honey, is consumed to treat migraine. A rag, soaked in the boiling water of the bark, is put on the forehead to treat cooling diseases. The bark is cut into small pieces, reduced to powder, mixed with *Citrus* × *limon* juice and honey, and eaten to cure fever. The bark juice is drunk twice a day for 3 days in case of gastric infections.	[16,20,26]	Skin diseases, hysteria, rheumatic pain [16], diarrhoea, constpation, cholera [23]
Toona hexandra (Wall.) M. Roem. (= *Toona ciliata* M. Roem.) GA20171101NP022	Tuni (T)	W	Wood	handicr	Wood is used for the production of furniture.	[16,17]	Infantile dysentery, ulcer and boils [16]
Menispermaceae							
Stephania glandulifera Miers GA20171110NP037	Gundri gano (T)	W	R	med/gast,mat,ant	Root juice is drunk once a day (morning or evening) to treat gastritis, menstrual disorders, and food poisoning.		Cough [27]
Tinospora sinensis (Lour.) Merr. GA20181015NP081	Gurjo (N)	W	St	med/genh,card	Stem juice or pieces of it are taken in case of cancer and piles.		Menstruation problems [20], diarrhoea, dysentery, stomachache [23]
Moraceae							
Ficus racemosa L. GA20181013NP068	Dumri (N)	W	Fr Lx	med/card med/derm	Ripe fruits are eaten to treat blood circulation disorders. Latex is applied to the skin affected by dermatological diseases.	[27]	Diarrhoea [26]
Ficus semicordata Buch.-Ham. ex Sm. GA20171110NP034	Ngedhore (T)	W	Br	med/gast	The bark of stem portion near the ground is crushed and boiled in a copper pot for about 3 h, adding small pieces of copper. The filtrate is drunk adding honey, twice a day, to treat dysentery with blood in the stool.		Scabies [25], wounds [26]

Table 2. *Cont.*

Family Scientific Name Voucher Number	Vernacular Name [a]	Origin [b]	Parts Used [c]	Ethnobotanical Uses [d]	Uses Description	Similar Uses References	Other Uses References
Musaceae							
Musa x *paradisiaca* L. GA20171101NP020	Kera (N)	C	Fr	food	Ripe fruits are edible and green bananas are cooked like vegetables.	[16,26]	Intestinal disorders, diabetes, uremia, nephritis, gout, hypertension, cardiac diseases [16], jaundice [27]
Myricaceae							
Myrica esculenta Buch.-Ham. ex D. Don GA20181014NP072	Kaphal (N)	W	Br	med/nerv,ENT,derm,resp	Dry bark powder is used in various remedies: it is inhaled 3 times a day to treat headache; in case of toothache, the teeth are washed with the mixture of bark powder and *Citrus* × *limon*; to treat earache, the bark powder is mixed with hot rapeseed oil to make an ointment for earache; to treat skin problems, the bark powder is applied to the skin with a "Nepali paper" bandage. For the treatment of sore throat the fresh bark is cut into small pieces which are placed inside the leaves of *Piper betle* ("paan") and eaten like candy.	[19,27]	Constipation [17], diarrhoea, asthma [19], bronchitis [24], cholera [26]
Myrtaceae							
Psidium guajava L. GA20181016NP092	Amba (T), Guava (I)	N	Br	med/gast	The bark juice from the stem portion near the ground is drunk to treat severe belly pains with blood in the stool.	[16,20,23,26,27]	Skin problems, rheumatism, cholera, headache [16], anthelmintic [23],blood pressure control [25]
Syzygium cumini (L.) Skeels GA20181015NP080	Jamuna (N)	W	Fr	med/met	The dried ripe fruit powder is diluited with water and drunk twice a day to control diabetes.		Diarrhoea [23], typhoid [26]
Oleaceae							
Jasminum mesnyi Hance GA20171113NP050	Ghyi fui (N), Gaiful (T)	N	R/Wp	med/fev	Root juice is taken once a day for 3 days to cure fever and the juice of the whole plant is used to cure typhoid.		
Nyctanthes arbor-tristis L. GA20181014NP074	Parijat (N)	W	L	med/fev	Leaf juice is drunk to cure fever.		Cold/cough [26,27]
Oxalidaceae							
Oxalis corniculata L. GA20181018NP114	Pang qui, Nakbruigumba (T), Pauja gai (Ne)	W	Wp	med/musc,fev,ant,nerv	Plant juice is drunk to treat joint pain and internal fever. A typical recipe (*) is used to cure fever, internal fever, food poisoning, joint pain, migraine.	[23,26]	Cataract [24], sinusitis [25], conjunctivitis, typhoid [26]

Table 2. *Cont.*

Family Scientific Name Voucher Number	Vernacular Name [a]	Origin [b]	Parts Used [c]	Ethnobotanical Uses [d]	Uses Description	Similar Uses References	Other Uses References
Phyllanthaceae							
Phyllanthus emblica L. GA20171101NP021	Amala (N)	W	Fr	med/resp	The fruits are consumed as expectorants in case of cough and sore throat.	[23,26,27]	Hearth pain, constipation, diarrhoea [23], gastritis [26]
Piperaceae							
Piper betle L. GA20181014NP077	Paan (N)	W	L	med/resp	The leaves of *Piper betle* ("paan") are used, in case of sore throat, to envelop small pieces of bark of *Myrica esculenta*, and therefore taken like candy.		
Piper retrofractum Vahl GA20171110NP036	Pan gughi (T)	N	St	med/gast	The stem, fresh or dried, is used for the treatment of gastric disorders. The stem is chewed or pounded to obtain a juice to drink.		
Plumbaginaceae							
Plumbago zeylanica L. GA20171111NP043	Chitu (N), Ping chittu (T)	W	Wp L	med/gast food	Plant juice is drunk, twice a day for a week, to treat gastric disorders. Young leaves are cooked in rapeseed oil.	[23]	Skin diseases [23], retention of urine [26]
Poaceae							
Eulaliopsis binata (Retz.) C.E.Hubb. GA20171101NP018	Arkhen khar (N)	W	Wp	dom	The dried plant is used as thatching roof.	[16]	
Oryza sativa L. GA20181016NP091	Sun (T), Nalasun (Ne)	C	Fr Ys	food med/fev,ant,musc,nerv	Rice is used in the preparation, along with *Blumea aromatica* and *Clematis buchananiana*, of the "chhaang" alcoholic beverage. A typical Newar recipe (*) is used to cure fever, internal fever, food poisoning, joint pain, migraine.	[16]	Hearth inflammation, indigestion [16]
Saccharum officinarum L. GA20171109NP026	Ukhu (N)	C	St	food med/urigen	Stem is sucked like candy or crushed to extract the sweetened juice. The stem, preferably the portion closest to the ground, is chewed when the bladder feels swollen and the urine is dark yellow.	[16]	Jaundice, stomach disorders, skin ulcers, seminal weakness [16]
Zea mays L. GA20181016NP094	Makai (N)	C	Fr	vet	Corn flour is mixed with *Asparagus racemosus* root juice and the mixture is given to buffaloes for about 2–3 days to stimulate milk production.		
Polypodiaceae							
Nephrolepis cordifolia (L.) C. Presl GA20181014NP073	Tui amala (T)	W	R	food	Watery root tubers are eaten as a snack to reduce thirst.	[24]	Bone fractures [26]

Table 2. *Cont.*

Family Scientific Name Voucher Number	Vernacular Name [a]	Origin [b]	Parts Used [c]	Ethnobotanical Uses [d]	Uses Description	Similar Uses References	Other Uses References
Pteridaceae							
Hemionitis anceps (Blanf.) Christenh. (=*Cheilanthes anceps* Blanf.) GA20171111NP040	Rani sinka (T)	W	St/L	med/gast	The plant is cleaned, boiled for about 10 min and consumed 3 times a day (morning, afternoon, evening) for 5–6 days, 10 days maximum,in case of stomach problems.		
Ranunculaceae							
Clematis buchananiana DC. GA20181015NP086	Chyanmangre (T)	W	St/R Wp	med/resp,gast food	The plant is crushed, wrapped in a cloth, and inhaled to treat sinusitis and allergic rhinitis. Root juice is drunk to cure gastric problems. The plant mixed with *Blumea aromatica* and *Oryza sativa*, is left to ferment for a week, obtaining a popular alcoholic beverage, "chhaang".		
Rosaceae							
Rubus ellipticus Sm. GA20181014NP078	Aniselu (N), Polang (T)	W	R Wp	med/resp,gast med/derm	The hand-crushed root is inhaled to treat rhinitis and sinusitis. The root juice is taken for gastric problems. The crushed plant, mixed with *Osbeckia nepalensis*, is applied on skin infections.	[19,24–27]	Typhoid [19], fever [24,25]
Rutaceae							
Aegle marmelos (L.) Corrêa GA20181012NP056	Bel (N)	W	Fr	food med/gast,fev	Ripe fruits are consumed or mixed with cold water to prepare a refreshing drink (*sarbat*). Ripe fruits are consumed to treat diarrhoea and fever.	[20,23,26]	Scabies and roundworm [23], diabetes [24]
Boenninghausenia albiflora (Hook.) Rchb. ex Meisn. GA20171111NP039	Thangkap mra (T)	W	Wp	med/fev med/nerv	Plant juice or boiled plant is taken 2–3 times a day (preferably in the morning) in the treatment of fever, until remission of symptoms. In case of headache, the crushed plant is applied on the forehead, inhaled, or fumigated.		Cold, insect repellent [27]
Citrus × *limon* (L.) Osbeck GA20181013NP062	Nibuwa (T)	C	Fr	food med/ant,ENT,fev	It is used as a flavoring. The *Citrus* × *limon* juice, mixed with the dry and pulverized bark of *Bauhinia variegata*, is applied on the snake bites and the part is bandaged. Lemon juice, mixed with the dry and pulverized bark of *Myrica esculenta*, is used to clean the teeth in case of toothache. Lemon juice, mixed with the powder of *Melia azedarach* bark, is used for the treatment of fever.		Cholera [26]

Table 2. *Cont.*

Family Scientific Name Voucher Number	Vernacular Name [a]	Origin [b]	Parts Used [c]	Ethnobotanical Uses [d]	Uses Description	Similar Uses References	Other Uses References
Citrus × *sinensis* (L.) Osbeck GA20171111NP041	Junar (T)	C	Fr	food med/gast,urogen	The ripe fruits are eaten, sometimes with chilly pepper. In case of hepatic and renal stones, 1–2 fruits are eaten a day, in small pieces during the day, for 15/25/30 days.		
Santalaceae							
Osyris lanceolata Hochst. & Steud. GA20171110NP035	Borsajini (T)	N	St St/Br	food med/musc	The stem portion near the ground is used to prepare a tea-like beverage. Used for dislocations and limb sprains. The stem portion near the ground is cut into small pieces, boiled for more than 3 h adding water; the filtered liquid is applied on the limbs banded with "Nepali paper". The bandage is changed regularly for about one month. In case of bone fractures, the bark is cut into small pieces, boiled (even for more than 3 h), sometimes together with bark of *Senegalia catechu* and *Albizia julibrissin*, applied on the injured part and banded with "Nepali paper".	[17,19]	Maternity problem [19]
Viscum articulatum Burm. f. GA20171109NP028	Khakhre bali (T)	W	Wp	med/musc	The crushed plant is applied on bone fractures and the part is banded with strips obtained from the *Urtica dioica* stem.	[24]	
Sapotaceae							
Diploknema butyracea (Roxb.) H.J.Lam GA20181015NP089	Chyuri (N)	W	Fr Br	med/derm,vet,fev	The crushed ripe fruits are applied externally for skin problems in humans and cattle. The bark of stem portion near the ground is dried, reduced to powder, mixed, and drunk with water or milk, to cure fever.		
Saxifragaceae							
Bergenia ciliata (Haw.) Sternb. GA20181017NP109	Pashanved (N), Bra mindhu (T)	W	Rh	med/gast,fev	Rhizome juice is drunk to treat stomach problems or the dried rhizome is chewed like candy throughout the day also to cure fever.	[16,19,24]	Piles, tumor, urinary problems, hearth, and respiratory diseases [16], maternity problem [17], back pain [25]
Simaroubaceae							
Picrasma quassioides (D. Don) Benn. GA20181018NP115	Nim kath (N)	W	Wood	med/fev,ant,musc,nerv	A typical recipe (*) is used to cure fever, internal fever, food poisoning, joint pain, migraine.		

Table 2. *Cont.*

Family Scientific Name Voucher Number	Vernacular Name [a]	Origin [b]	Parts Used [c]	Ethnobotanical Uses [d]	Uses Description	Similar Uses References	Other Uses References
Solanaceae							
Solanum nigrum L. GA20181012NP053	Camai (T)	W	Fr	med/ENT,card	Ripe fruits are consumed as much as possible, in case of tongue infections and piles.		Headache [23], wounds [24], malnutrition in children [26]
Thymelaceae							
Daphne bholua Buch.-Ham. ex D. Don GA20171110NP031	Lokta (N)	C	Br	handicr med/derm,ant,ENT,musc	The bark is used to make "Nepali paper". "Nepali paper" is used to make bandages in case of skin problems, snake bites, bone fractures, swelling of the dental glands.	[16]	Fever, intestinal disorders, and parasites [16], sinusitis [19]
Urticaceae							
Boehmeria virgata (G.Forst.) Guill. subsp. *macrophylla* (Hornem.) Friis & Wilmot-Dear. (=*Boehmeria macrophylla* Hornem.) GA20171101NP016	Chalnesisnu (N)	W	L	vet	Leaves are nutritious cattle fodder.	[16]	Cuts and wounds [16,23]
Boehmeria rugulosa Wedd. GA20171101NP015	Bhlan chhing (T)	W	Br Wood	food handicr	Powdered bark is mixed with flour to make the bread softer and tastier. Wood is used for the production of religious masks and *teki*, container where butter (*ghee*) and yogurt are prepared.	[16]	Cuts and wounds, body pain [16]
Urtica dioica L. GA20171109NP027	Sisnu (N)	W	St	med/musc	Some strips obtained from the stem are used to wrap the limbs affected by bone fracture in association with the *Viscum articulatum*.	[17,26,27]	Galactogogue, diabetes, high pressure [17], fever, asthma, toothache, paralysis, uterine bleeding [19], rheumatism [25]
Verbenaceae							
Lantana camara L. GA20171101NP019	Polung (T)	N	Fr	food	Ripe black fruits are eaten by children as snack.		
Xanthorrhoeaceae							
Aloe vera (L.) Burm.f. GA20171109NP024	Ghyukumari (N)	C	L Fl	med/derm,met,gast dom	The leaves are rubbed on burned skin. To treat jaundice, the leaf juice, or the filtered liquid of the leaves, crushed and boiled for 2–3 minutes is drunk. Some pieces of leaves are eaten in case of lack of appetite. Flowers are used as an ornamental decoration.	[19,20,26,27]	Cuts and wounds [23,24]

Table 2. *Cont.*

Family Scientific Name Voucher Number	Vernacular Name [a]	Origin [b]	Parts Used [c]	Ethnobotanical Uses [d]	Uses Description	Similar Uses References	Other Uses References
Zingiberaceae							
Cautleya spicata (Sm.) Baker GA20171110NP030	Pahelo Ausadhi (N) Jungli haldi (T)	W	Rh	med/gast,urogen	Rhizome juice is drunk once a day (morning) to treat constipation (*kabjiat*) and kidney stones. In case of gastric disorders, the juice is drunk once a week. Sometimes a second dose may be needed.		Conjunctivitis [26]
Curcuma angustifolia Roxb. GA20181013NP063	Jangali Haldi, Jangali Besar (N)	C	Rh	med/resp	Fumigations with powdered rhizome are useful to cure cold.		Cuts and wounds [24], stomach problems [26]
Curcuma caesia Roxb. GA20181017NP102	Mlang haldi (T)	C	Rh R	med/gast,mat	Used in case of loss of appetite. The cleaned rhizome is crushed and the juice is drunk with the addition of water, twice a day for 2 days. The root juice is drunk to treat postpartum bleeding.		Back pain [25]
Curcuma longa L. GA20181013NP064	Haldi, Besar (N)	C	Rh	med/met,fev	The raw rhizome is eaten to cure diabetes. The powdered rhizome is used for fumigations in case of fever.		Cough/cold, tonsillitis, swellings [16], gastritis [25]
Zingiber officinale Roscoe GA20181015NP083	Aduwa (N)	C	Rh	med/met	To cure goiter, the dried rhizome powder is mixed with the *Bauhinia variegata* bark powder and water, and applied to the throat twice a day (morning and evening).		Diarrhoea, sinusitis [23], cold and cough [27]

[a] N—Nepali; Ne—Newari; T—Tamang; [b] W—wild; C—cultivated; N—naturalized; [c] Br—bark; L—leaves; Fl—flowers; Fr—fruit; Ys—young shoots; Lx—latex; R—root/rhizome; Sd—seeds; St—stem; Wp—whole plant; [d] agr—agriculture; dom—domestic use; handicr—handicraft; med—medicinal use; ant—antidote; card—cardiovascular; derm—dermatological; ENT—oral dental ENT; fev—fever; gast—gastrointestinal; genh—general health; mat—maternity; met—metabolic; musc—musculoskeletal; nerv—nervous system; resp—respiratory; urogen—urogenital; vet—veterinary; * Typical mix of plants: *Centella asiatica*, *Tagetes erecta*, *Mentha spicata*, *Artemisia indica*, *Oroxylum indicum*, *Oryza sativa*, *Picrasma quassioides*, *Oxalis corniculata*, *Drymaria cordata*.

APPPENDIX IV

Figures showing optical and electronic microscopic analyses of *Valeriana jatamansi*, *Nardostachys jatamansi* and *Valeriana officinalis* (Cornara et al., 2020).

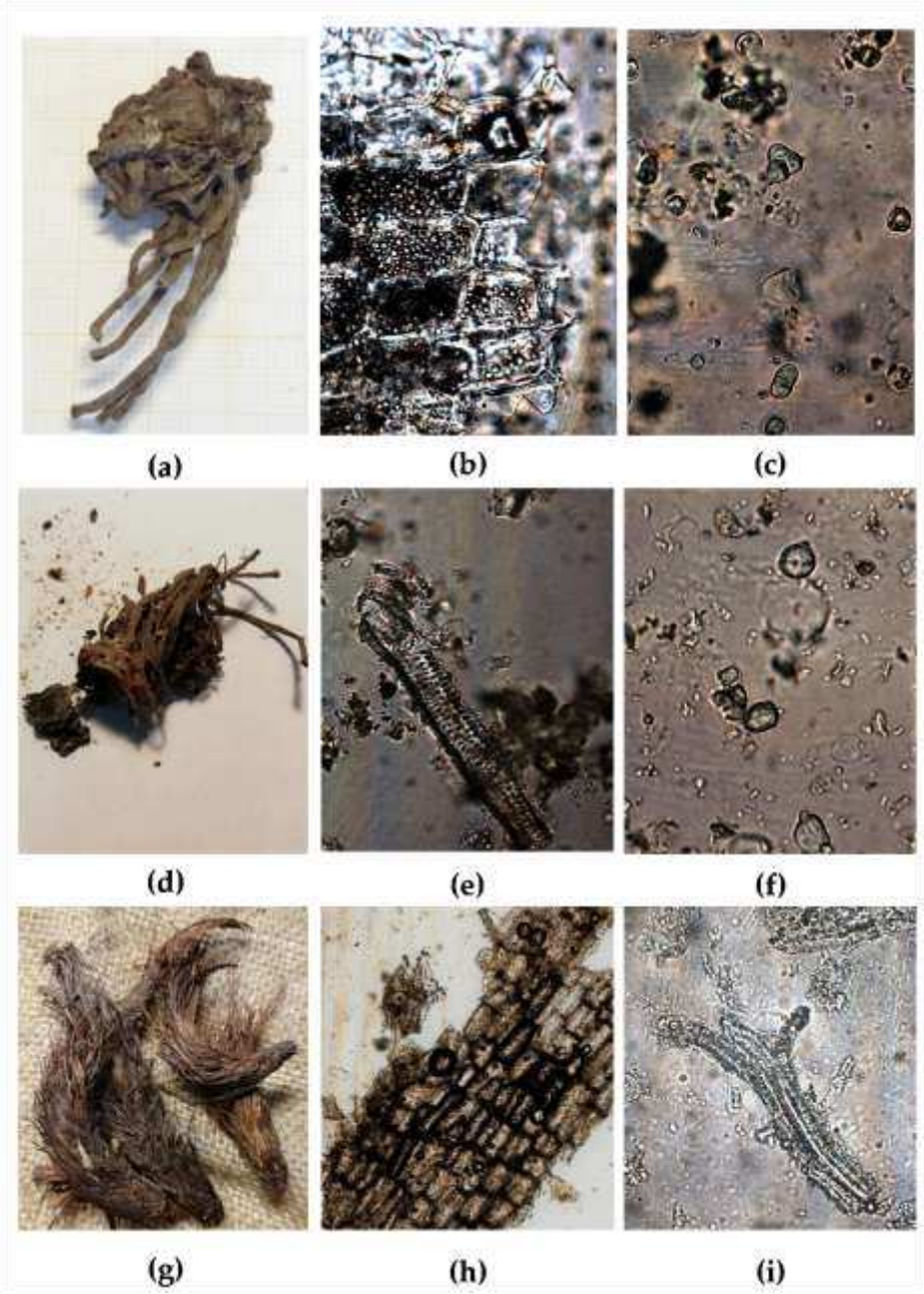

Figure 1. (a-c) Vo: (a) dried root/rhizome; (b-c) powdered material: (b) parenchymatous cells with starch grains (20x); (c) starch grains (40x); (d-f) Vj: (d) dried root/rhizome; (e-f) powdered material: (e) scalariform vessels (20x); (f) starch grains (40x); (g-i) Nj: (g) dried root/rhizome; (h-i) powdered material: (h) cork cells (10x); (i) fibers and small starch grains (40x).

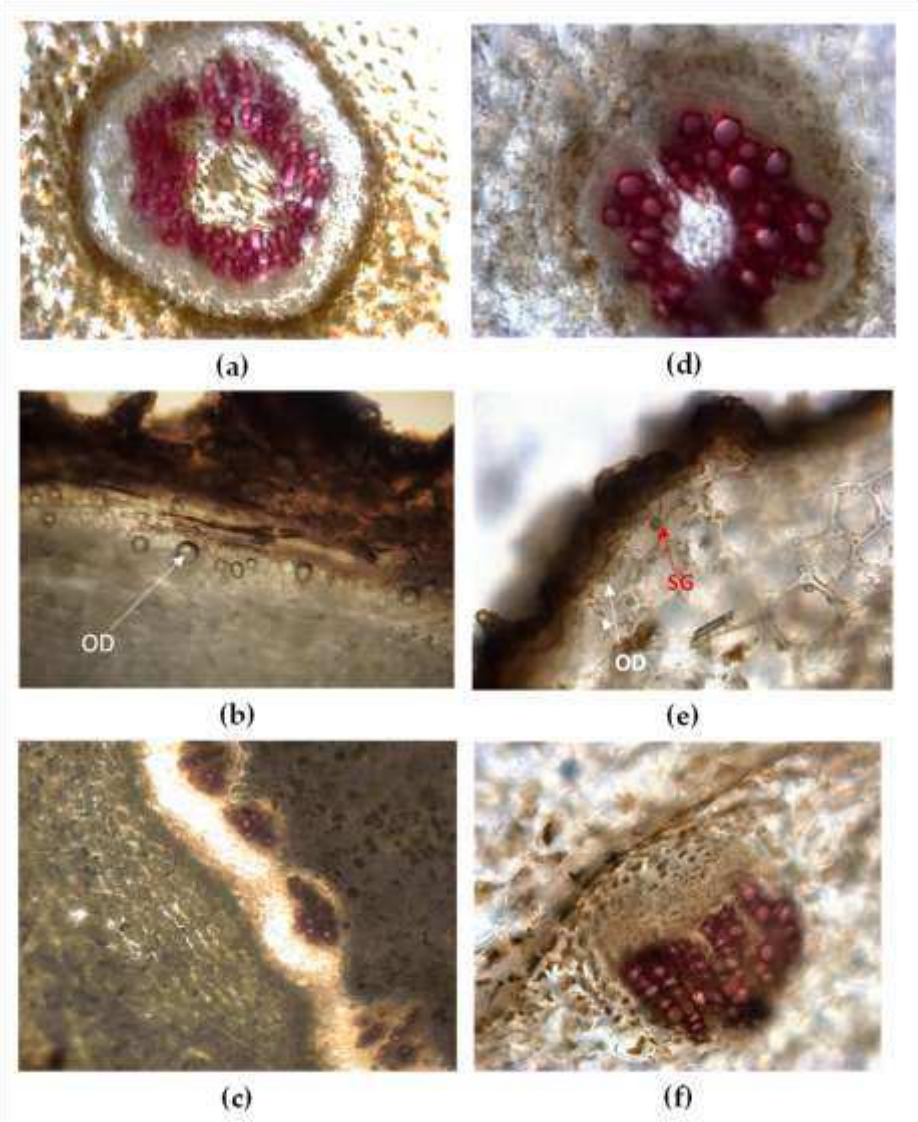

Figure 2. Optical microscopy view of root/rhizome TS of Vo (a-c) and Vj (d-f) stained with phloroglucinol-HCl. Vo: (a) cortex and stele in an older root; (b) under the rhizome cork the outermost layers of parenchymatous cortex contain oil globules; (c) collateral vascular bundles circularly arranged in the rhizome. Vj: (d) cortex and stele in an older root; (e) starch grains and oil globules are visible within parenchymatous cortex; (f) magnification of a singular collateral vascular bundle in the rhizome.

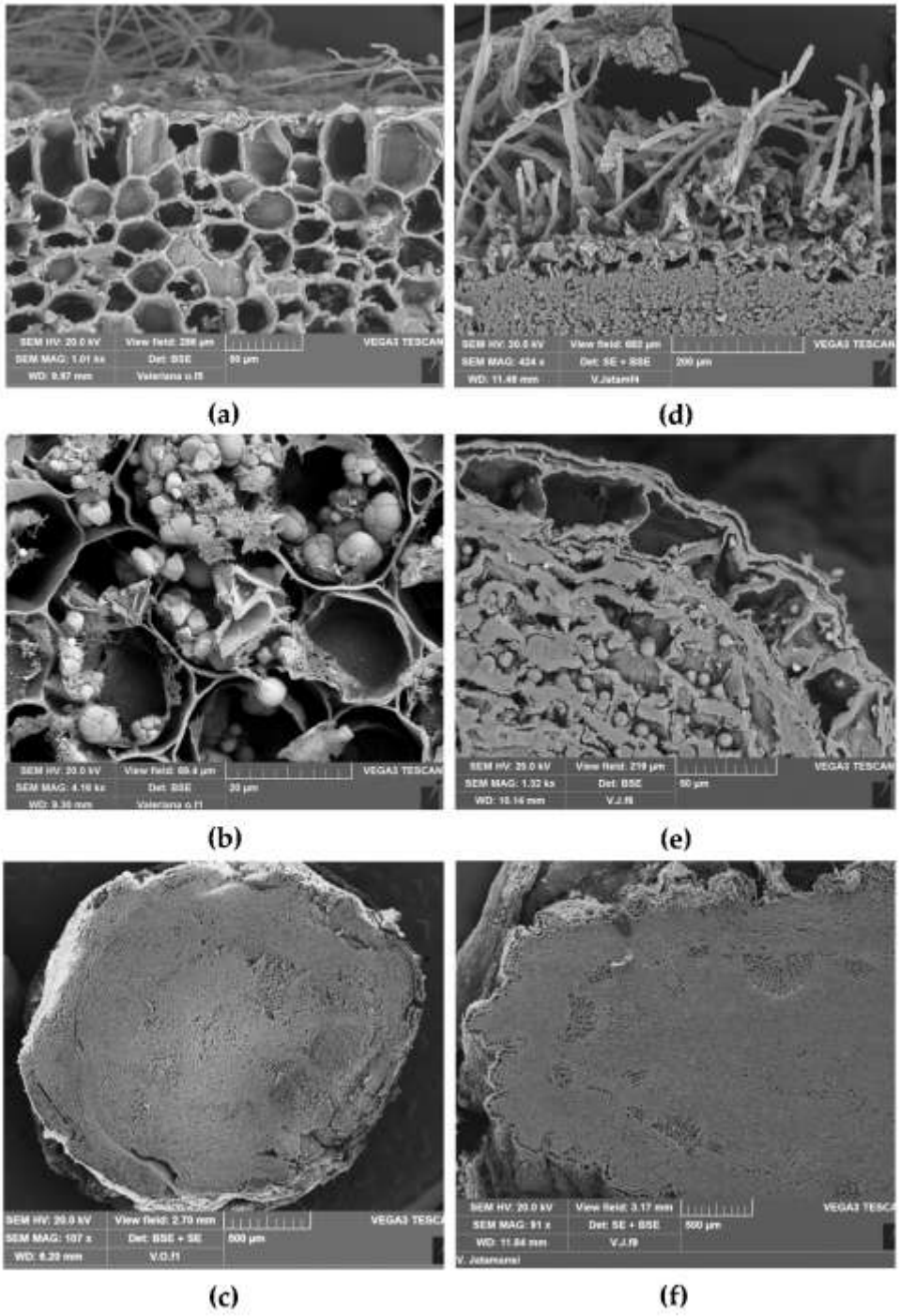

Figure 3. Scanning electron microscopic view of TS of root/rhizome from Vo (a-c) and Vj (d-f): (a) epidermis with root hairs and the hypodermal layer of the cortex; (b) starch occurring as single or compound grains (2-6 components) within cortical parenchymatous cells; (c) TS of rhizome showing vascular bundles circularly arranged; (d) epidermis with many root hairs and parenchymatous cells filled with starch grains; (e) exoderm and parenchymatous cells filled with starch, generally occurring as single or compound grains with two components; (f) TS of rhizome with many vascular bundles surrounding the central pith.

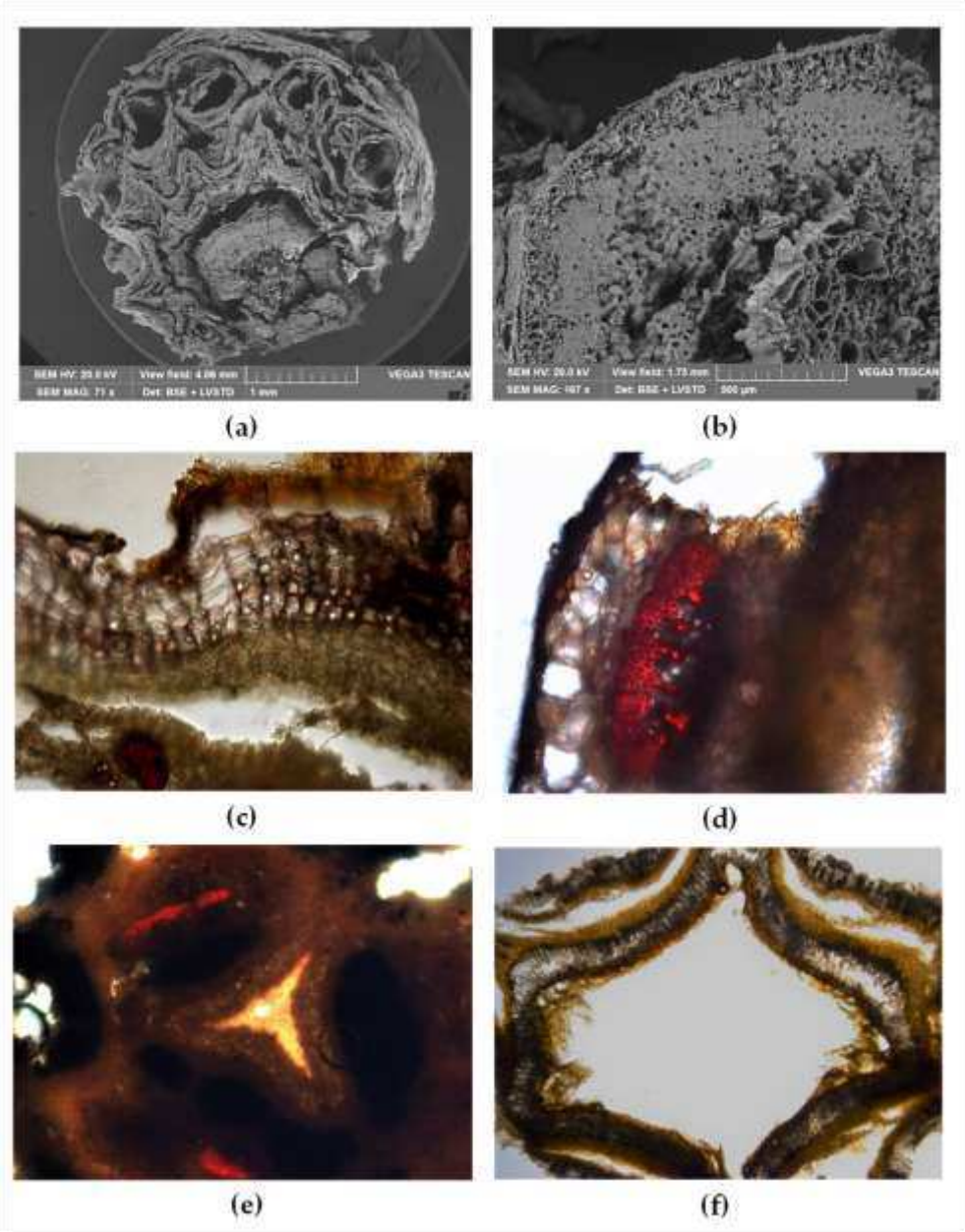

Figure 4. Nj: (a-b) SEM micrographs, (c-f) MO observation of TS rhizome stained with phloroglucinol-HCl. (a) The rhizome is surrounded by many remains of the basal leaves petioles; (b) particular of TS rhizome in which a multi-layered cork and large bundles of sclerenchymatous fibers are visible; (c) within suberized cork cells many oil globules are present; (d) bundles of sclerenchymatous fibers appear red-stained by phloroglucinol-HCl; (e) the parenchymatous pith showing a characteristic subtriangular-stellate shape is enclosed by cork rings; (f) older portion of the rhizome shows a necrotic pith, the cavity of which is surrounded by medullary cork layers.