Contents lists available at ScienceDirect



European Journal of Integrative Medicine

journal homepage: www.elsevier.com/locate/eujim



Research paper

An integrated medicine of Bhutan: *Sowa Rigpa* concepts, botanical identification, and the recorded phytochemical and pharmacological properties of the eastern Himalayan medicinal plants



Karma Yeshi^a, Yangbum Gyal^b, Katharina Sabernig^c, Jigme Phuntsho^d, Tawni Tidwell^e, Tenzin Jamtsho^f, Rinchen Dhondup^g, Eliot Tokar^h, Phurpa Wangchuk^{i,*}

^a Sherubtse College, Royal University of Bhutan, Trashigang District, Bhutan

^b Cultural Linguistic Services, University of Wisconsin-Madison, USA

^c Medical University of Vienna, Austria

^d Sherubtse College, Kanglung, Trashigang District, Bhutan

^e Institute for Social Anthropology, Austrian Academy of Sciences, Vienna, Austria

^f Yangchenphug Higher Secondary School, Thimphu District, Bhutan

⁸ Tibetan Medical College, Qinghai University, Xining, China

^h Eliot Tokar- Tibetan medicine, NYC, USA

ⁱ Australian Institute of Tropical Health and Medicine, James Cook University, Cairns Campus, QLD 4870, Australia

ARTICLE INFO

Keywords: Integrative medicine Sowa Rigpa concepts Pharmacological activities Ethnotaxonomy Eastern Himalayas Bhutan

ABSTRACT

Introduction: Bhutanese *Sowa Rigpa* medicine (BSM) has established unique pharmacopoeias that describe ethnotaxonomical concepts, classification systems and descriptions of medicinal plants. BSM was integrated with modern health care system in 1967. BSM uses medicinal plants, minerals and animal products as the main ingredients of the formulations used for treating various disorders. This study was aimed to highlight interesting ethnotaxonomical concepts of BSM and identify and document medicinal plants from the two remotest regions of eastern Himalayas of Bhutan – Merak and Sakteng.

Methods: Ethnopharmacological data was gathered from the pharmacopoeias, published monographs and research papers on Bhutanese medicinal plants. Medicinal plants diversity was surveyed using the convenience sampling methods. A literature review on the phytochemical content of the medicinal plants and their pharmacological activities was carried out using plant databases, PubMed, SciFinder and Google Scholar.

Results: A very interesting ethnotaxonomical concept of BSM has been translated into English terminologies from the traditional pharmacopoeia. A total of 50 alpine medicinal plants belonging to 25 families and 39 genera in 13 study sites between Merak and Sakteng were documented. Only 12 medicinal species were identified as priority species or are currently used at Menjong Sorig Pharmaceuticals for formulating 48 different medicinal plants in Bhutan and botanically identified 50 alpine medicinal plants from 13 different study sites under Merak and Sakteng regions. About 12 of them were currently used as ingredients in BSM formulations and 35 species were studied for their phytochemical and pharmacological properties.

1. Introduction

Sowa Rigpa (Wylie transliteration of Tibetan, gso-ba rig-pa or "knowledge field of healing"), also known as Tibetan medicine, is a scholarly Asian traditional medical system rigorously transmitted through canonical text and oral teachings. It is widely known in the

West as Tibetan medicine, and is practiced worldwide especially in countries such as in Tibetan regions of China, Himalayan regions of India (Ladakh, Sikkim, Himachal Pradesh), Nepal, Bhutan, Mongolia, Russia, and recently in the European and North America countries such as Switzerland, Germany, Canada, United States, and Mexico [1]. This medical system has a uniquely codified and well-documented *materia*

* Corresponding author at: AITHM, James Cook University, Cairns, Australia.

E-mail addresses: karmayeshi@live.com (K. Yeshi), yangbum.gyal@wisc.edu (Y. Gyal), katharina.sabernig@meduniwien.ac.at (K. Sabernig), jigme024@gmail.com (J. Phuntsho), tawni.tidwell@gmail.com (T. Tidwell), jamtshooo@gmail.com (T. Jamtsho), rqdd@yahoo.com (R. Dhondup), etokar@aol.com (E. Tokar), phurpaw@yahoo.com (P. Wangchuk).

https://doi.org/10.1016/j.eujim.2019.100927

Received 21 May 2019; Received in revised form 10 June 2019; Accepted 11 June 2019 1876-3820/@ 2019 Elsevier GmbH. All rights reserved.

medica, extensive pharmacopoeia, prolific traditional and contemporary medical commentaries, and systematic disease classification systems, diagnostics and treatment regimens [2]. Historically, principles and techniques from other major traditional medical systems, including Ayurveda, Greco-Arabic and Chinese traditional medicine, were incorporated into the *Sowa Rigpa* medical system [3].

Sowa Rigpa (gso-ba rig-pa) developed its own canonical text, the Rgyud-bzhi or Four Tantras, a four-part text compiled in the twelfth century and still used centrally today. Historically, these four Tantras and their commentaries and other pharmacological medical texts form the basis in Sowa Rigpa medical teachings and trainings. The early eighteenth century pharmacopoeia Shel-gong shel-phreng authored by Bstan-'dzin-phun-tshogs is the most famous text (1673–1743) [4]. Based on this work, the Mongolian scholar' Jam-dpal-rdo-rje later edited his illustrated material medica including Tibetan, Mongolian, Manchu and Chinese [5]. In the meantime, a number of contemporary publications have been published on the Sowa Rigpa material medica. The 'Khrungs-dpe dri-med shel gyi me-long edited by Dga'-ba'i-rdo-rje [6] is one example that also found international recognition. While Shelgong shel-phreng [4] describes 2294 different single medicinal substance including their classification, description, nature, potencies and identifications; 'Khrungs-dpe dri-med shel gyi me-long [6] describes all three types of ingredients including plants, animals and minerals and provides nomenclature, classification, nature, potencies and identification with illustrations [3].

In Bhutan, the Bhutanese tradition of Sowa Rigpa was tailored to the country's needs by Bhutanese traditional physicians called Drungtshos (drung-'tsho: traditional physician), relying upon their rich regional biodiversity and the prevalence of diseases in their historic kingdom. The differences and similarities between the mainstream traditions of Tibetan and the Bhutanese Sowa Rigpa have been described elsewhere [2]. Sowa Rigpa in Bhutan is often known as indigenous medicine or the Bhutanese traditional medicine (BTM) or the Bhutanese Sowa Rigpa medicine (BSM). It was officially integrated with the modern health care delivery system in 1967 and today, the BSM and modern health care services are both patronized by the Ministry of Health in Bhutan and provide free health care services to people from the same hospitals and health care centres in the country [7]. Although BSM started with a small dispensary in 1967, it has expanded its services very rapidly and at present there are 70 BSM centres/units in the country including one National Traditional Medicine Referral Hospital (NTMRH, Thimphu), two Regional Traditional Medicine Referral Hospital (Mongar and Gaylegphu), 20 district hospitals and in the selected Basic Health Units.

The treatment regimens of BSM include change in food and lifestyle as many illnesses arise from improper diet and lifestyle, complex polyingredient medicinal formulations, and five kinds of external therapies namely bloodletting (*gtar*), gold/silver needle (*gser-khab/ dngul-khab*), heated oil cauterization (*snum-tshug*) and moxibustion (*mebtsa*), medicinal bath and steam (*rlang-dug/rlang-lum/chu-lums*), and nasal irrigation (*sna-sbyong*). More than 100 polyingredient formulations are manufactured at Menjong Sorig Pharmaceuticals (MSP) under strict Good Manufacturing Practices (GMP) regulations using minerals, animal and plant ingredients. MSP, which is the only state-owned pharmaceutical company in Bhutan (http://www.dtms.gov.bt/ menjong-sorig-pharmaceuticals/), was established in 1998 for the purpose of manufacturing traditional medicines and conducting scientific research to improve their quality and safety.

Medicinal plants are the bulk ingredients of BSM formulations and more than 200 medicinal plants are currently in use at MSP. BSM is rich in ethnotaxonomical nomenclature and has a rigorous classification system, which classifies the medicinal plants as *sngo-sman* or high altitude medicinal plants (HAMP, 2000–5000 meters above sea level, literally smaller plants, herbs) and *khrog-sman* or low altitude medicinal plants (LAMP, 100–1999 masl) [8,9]. The *sngo-sman* and *khrog-sman* concepts in mainstream Tibetan *Sowa Rigpa* Medicine (TSM) slightly differ from BSM, where in TSM, *sngo-sman* are plants in which all parts (roots, leaves, flowers, fruits) are used, and *khrog-sman* refers to the raw harvested plant substances before processing [6,4]. The HAMP in BSM are collected from the northwest high-altitude mountains of Lingzhi region (near the Tibetan border), and LAMP from the valleys of Langthel *rged-wog* (pronounced as 'Gewog' which is equivalent to 'block', an administratively demarcated region constituted by many villages) under Trongsa district in central Bhutan [10]. Other than the northwestern and south-central parts of Bhutan, no medicinal plant have been identified from the eastern regions of Bhutan. This raises many research questions including: 1) Do medicinal plants used in BSM grow in the eastern Himalayas of Bhutan? 2) If they do grow, at what altitudes do they grow? 3) What types of medicinal plants are available there? 4) What is their status of availability? 5) Could these plants be collected by farmers to earn extra income for their households?

To answer these questions, we first consulted BSM practitioners and MSP procurement officers, and then carried out medicinal plants surveys in two northeastern regions of Bhutan, called the Merak and Sakteng Gewogs (Blocks), in 2017 to botanically identify the medicinal plants. In addition, to provide a context for our botanical identification and descriptions of medicinal plants from those two regions, we have carried out literature reviews of traditional medical textbooks and the published scientific papers. We have described their ethnotaxonomical concepts of BSM and the phytochemical and the pharmacological properties of medicinal plants. For a resource-contraint country, conducting scientific studies and clinical trials on BSM plants and formulations are difficult. Since the plants that we have botanically identified here may grow in other parts of the Himalayas, it is likely that some of the plants may already have been scientifically studied elsewhere. To determine the pharmacological activity, safety and toxicity of medicinal plants, describing its conservation status as well as ecological value is important for BSM practitioners, patients, and policy makers in Bhutan. Therefore, we have compiled the existing literature on the phytochemical content and pharmacological activities of the medicinal plants identified here, as well as performed further content analysis. Such compiled data of existing scientific studies specific to the medicinal plants of Bhutan will inform future studies and analysis on the ethnopharmacology of BSM, as well as appropriately apprise policymakers, healthcare providers and patient population. The objectives and findings of this study are presented here in three main categories related to plant characterization: a) ethnotaxonomical concepts related to those species in the study recognized as medicinal by BSM, b) botanical identification of all surveyed plants growing in the north-eastern Merak and Sakteng regions, and c) a literature review of the existing phytochemical and pharmacological studies of the BSM medicinal plants in the study area.

2. Materials and methods

2.1. Study area, population and ethical consideration

The Merak and Sakteng regions are located in the alpine climatic zone with a mean temperature of 5.5°C and annual average rainfall of \leq 650 mm [11]. Merak and Sakteng are two of the 15 Gewogs under Trashigang district. Merak has a total land area of 867.7 sq. km and Sakteng has 910.9 sq. km [12] (Fig. 1). Unlike other Gewogs under the same district, these two Gewogs are inhabited by a unique ethnicity called Brokpa ('brog-pa, nomadic highlanders) and they have unique culture and traditions of their own. There are more than 2200 Brokpas inhabiting the Merak Gewog (comprising 260 households) and about 2600 Brokpas living in the Sakteng Gewog (comprising 389 households) [12]. We chose Merak and Sakteng as our study areas for the following reasons: a) Merak and Sakteng are unexplored areas for medicinal plant growth, b) the livelihood of Merak and Sakteng Brokpas are totally dependent on animal husbandry based on Yaks and the study could motivate their engagement in the sustainable medicinal plants collection program (priority species) as a good alternative to generate cash



Fig. 1. (A) Regional map showing Bhutan. (B) Map of Bhutan showing Trashigang District and the location of the two Gewogs with boundary demarcation. (C) Location of Merak and Sakteng with 13 study plots.

income, and c) MSP requires sustainable supply of medicinal plants to cater to 70 BSM centers wide across the country. So, expanding collection to Merak and Sakteng could contribute to supply. We covered 13 areas/locations within the Merak and Sakteng regions as listed below:

- Nyakchungla (3925-3965 masl)
- Drangmazur (3900–3990 masl)
- Tshonag (3810-3959 masl)
- Dangla (3915–4060 masl)
- Samnya (3880–3979 masl)
- Kamloong (3910-4010 masl)
- Sernyasa (3895–4100 masl)
- Tshogara (3995-4120 masl)
- Loombur (4000–4100 masl)
- Nagapa (3900-4025 masl)
- Kargyap (3985–4080 masl)
- Kayakpa (3500-4010 masl)
- Yanglay-yangchen (3800-4100 masl)

Although all study sites are not connected by motor road, listed medicinal plants are all one day walking distance from Gewog offices in Merak and Sakteng, with 6 to 7 h hike as the maximum distance traveled to reach a specimen site. The Merak Gewog office is connected by motor road while the Sakteng Gewog is not.

The medicinal plant diversity survey including collecting of plants, ethnopharmacological data mining, and compiling databases and images were undertaken in compliance with the International Society of Ethnobiology (ISE) code of ethics [13] and Access and Benefit Sharing (ABS) Policy of Bhutan 2015 [14]. The information on the uses of traditional knowledge found in the study sites are not documented in this study as it falls in the ambit of Access and Benefit Sharing (ABS) Policy of Bhutan 2015 [14]. No harmful consequences (biological or cultural) for the participants or local inhabitants found in the study area arose from this research and its related activities. All the ethical and informed consent to survey the research sites were obtained from Merak Gewog (block) head - Gup. Throughout conducting this research, all principles of the code of ethics [13,14] were adhered to including intellectual property rights and to secure the trusteeship of Bhutanese communities over their traditional knowledge associated with biological resources.

2.2. Study design, survey methods and team

We followed the review-based descriptive and observational study design previously described for the identification of plant-derived, animal-derived, and geomineral-derived pharmaceuticals used in BSM [9,3,15]. Briefly, we first collected, reviewed and evaluated the classical Tibetan Sowa Rigpa texts, and the BSM formulary books and monographs, which were used as references or teaching materials at the Faculty of Traditional Medicine (FoTM) of Khesar Gyalpo University of Medical Sciences of Bhutan, Department of Traditional Medicine Services (DTMS) of the Ministry of Health and MSP in Bhutan. We translated the ethnopharmacological uses of each plant ingredient into English medical terminologies with the help of drung-'tsho (Traditional physician trained for 5 years and 6 months including one year compulsory internship in the district hospital), sman-pa (clinical assistant trained for 3 years without internship) and other MSP research experts. See Table 4 for a summary of these translated uses and terminologies. To validate the textual descriptions, Drung-'tsho, sman-pa and a pharmacognosist were informally queried on the ethnotaxonomical concepts and classification system for medicinal plants.

Second, list of BSM medicinal plants was generated and then the field trip was made to the study area, the northeastern regions of Bhutan (Merak and Sakteng Gewogs) for field observations, photographing, herbarium specimen collections and other data collections. The medicinal plants were surveyed using the convenience sampling methods. Both traditional and botanical identification methods of medicinal plants were used during the field survey. Plants were first identified using traditional names and descriptions and then later botanically identified either at base-camp or upon returning to MSP using herbarium specimens, digital photographs of specimens, or other recorded field information such as habitat type, altitude, and plant life form. The location of specimen collection areas and altitudes were recorded using standard GPS altimeter units. Live plant specimens were collected, processed and deposited at the MSP herbarium centre for future reference. Using a series of original publications on the flora of Bhutan and other Himalayan regions [8,16-28] and the online databases such as 'The Plant List' (Version 1.1. 2013) [29], eFloras [30] and TROPICOS [31], we confirmed the identity of the medicinal plants that were growing in our study areas. The Sowa Rigoa names of the plants identified in the field were authenticated and confirmed with the help of Drung-'tsho at the National Traditional Hospital, and other experts at MSP. Local names (Brokpa kha) of the plants were written as per the pronunciation of local guides and local inhabitants of Merak and Sakteng.

The research team included one senior researcher from MSP (with extensive knowledge on botany and pharmacognosy), one botanist who is working under Ministry of Education of Bhutan, and one undergraduate student (with good background knowledge of botany from Sherubtse College of the Royal University of Bhutan). Two Brokpas (local inhabitants of Merak Gewog) served as local guides during our entire survey. We have informally gathered information from these two local guides about the local Brokpa (vernacular) names of the 50 plant species recorded from two Gewogs. However, local inhabitants (Brokpas) were not interviewed on the medicinal plants described in the Sowa Rigpa texts as most of them lacked knowledge about these species. The medicinal plants that are used by the local healers in the two communities of Merak and Sakteng were not allowed to document in this study since their oral traditional knowledge are not yet in the public domain and therefore their intellectual property rights are currently being protected by the National Biodiversity of Bhutan.

2.3. Criteria setting and data analysis

All medicinal plants identified in this survey were described with their Sowa Rigpa medicine names (transliterated), botanical name, family, part used and their ethnomedical uses. Status for each plant species was assigned as 'rare', 'available' and 'abundant' based upon the number of counts or citations in the areas covered by the study as described earlier in [8]. The plants with less than 10 counts or citations in the study areas covered by our survey were scored as 'rare' or 'limited' in their number, with 10-50 counts or citations as 'moderately available' and those with more than 50 as 'abundantly available'. All information such as habitat, life form and altitude are recorded in situ. We listed all the medicinal plants currently used by MSP and analysed the BSM formulations for the use frequency of each plant, along with their use frequency ranking. The entire information gathered from the study areas by the research team during the field survey were analysed using MS excel sheet and interpreted into tables, graphs and statistical graphics.

2.4. Literature review of medicinal plants for phytochemical content and their pharmacological activities

The BSM characterizes the quality of polyingredient formulations depending upon the quality of their individual ingredients. Therefore, MSP has strategically focused on scientifically validating the ethnopharmacological uses of individual ingredients. MSP conducted a series of phytochemical and pharmacological activity screenings from 2002 to 2016. Numerous studies on Himalayan medicinal plants used in *Sowa Rigpa* medicine have been conducted by other researchers from Nepal, Tibet (China), India and Switzerland among others. In this study, we reviewed published scientific articles on phytochemical and biological activities of the 50 Himalayan medicinal plants that we identified from

the northeastern Bhutanese Himalayas. We used Google Scholar, PubMed, SciFinder Scholar and plant databases to mine the published literature and tabulated the findings.

3. Results

3.1. Ethnotaxonomical concepts and the classification of medicinal plants in Bhutan

3.1.1. Indigenous descriptions of a plant

We found that that *Drung-'tshos* and *sman-pas* describe, define, name, identify and classify medicinal plants in their local terms and nomenclature (mostly Bhutanese Dzongkha language and classical Tibetan texts) based on the altitude, habitat, morphology, uses, cultural and religious significance, metaphorical terms, and organoleptic properties of the plant. *Drung-'tsho* in BSM primarily distinguish eight main parts of a plant which slightly differs from original Tibetan *Sowa Rigpa* texts such as *Rgyud-bzhi: rtsa-ba* (root), *sdong-po* (stem/trunk), *yal-ga* (branch), *pags-ko* (bark), *nyu-gu* (shoot), 'dab-ma (leaf), *me-tog* (flower) and 'bras-bu (fruit). In *Rgyud-bzhi*, they are categorised as: *rtsa-ba* (root), *sdong-po* (trunk), *yal-ga* (branch), *lo-ma* (leaf), *zho/tshi-ba/thang-chu* (milk/pitch/sap), *myu-gu* (buds of shoots), *me-tog* (flower), 'bras-bu (fruit), *rtse-mo* (tip), and *shun-pa* (bark).

3.1.2. BSM grouping and medicinal plant classification

According to the BSM physicians, all medicinal plants can be grouped under the broad category called shing (literally wood or tree, in this context plant kingdom). Shing is divided into two main divisions: me-tog can gi shing (flowering plants) and me-tog med-pa'i shing (nonflowering plants). BSM further sub-divide these two major divisions/ groups into six orders as sa-gter sngo-shing (herbaceous plants), sdong-po can gyi shing (erect plant with woody trunk or stem), tsher-ma can gyi shing (plants with thorns), khri-shing (climber), rtswa (grass) and sha-mu dang' jar-hon gyi rigs (fungi and lichens). The medicinal plants belonging to these six orders are then segregated into three main categories based on the altitudes they grow at. As mentioned above they are sngo-sman (high altitude medicinal plants) and khrog-sman (low altitude medicinal plants), additionally there is a category called rtsi-sman difficult to translate, often defined as resin or mucilaginous substances [32]. The sngo-sman is generally considered herbaceous and grows in the alpine snowy mountains in an altitude range of 2000 to 5000 m above sea level (masl). The khrog-sman is mostly woody types and grows in the temperate and tropical zones at an altitude range of 100 to 1999 masl. The rtsi-sman, although considered a separate class, is mostly extracted from the LAMPs and seldom from HAMPs. These three categories are further sub-categorized into seven origins based on the seven habitats types, including nags-tshal (forests), spang-gzhi (grassland), la/gangs-ri (mountain tops), lung-pa (enclosure of valleys), rtswa-'brog (cultivable dry lands), chu-zhing or 'dam-rdzab-can (marshy or wetlands), and brag (rocky cliffs).

Medicinal plants of these seven origins are divided into various rigs (Genus). Since the plants under same rigs are related morphologically, it correlates to the botanical class of a 'genus'. For example, medicinal plants constituting the type ba-lu-rigs showed a relevance to the genus Rhododendron, while the type bong-nga-rigs correlated to the genus Aconitum. Other types include dbyi-mong-rigs (species from a genus Clematis), kyi-lce-rigs (species from a genus Gentiana), utpal-rigs (species from a genus Meconopsis), and skyer-pa-rigs (species from a genus Berberis). Each type is graded as a sman-mchog (superior quality) or of sman-dman-pa (inferior quality). When there is a shortage of the smanmchog, sman-dman-pa or similar sub-types are often used as substitutes for preparing the polyingredient formulation. Based on their uses and properties (nus-pa chags-'tshal), medicinal plants of various types are also named and sub-categorized into different utility groups (sbyor-rigs) as: 1) spos-rigs (incense type) used for making incense products; 2) dugrigs (poisonous type) used as medicine after its detoxification; and 3)



Fig. 2. Medicinal plants named after the color of their flowers (courtesy: Phurpa Wangchuk collection): a) Rhododendron Anthopogon named as dwa-li-dkar-po, b) Cavea tanguensis as ming-can-nag-po, c) Rhodiola crenulata as sro-lo-dmar-pa, d) Inula grandiflora as ming-can-ser-po, e) Meconopsis simplicifolia as utpal-sngon-po, f) O. hookerias' bri-mog.

thang-rigs (neutralizer type) used for neutralizing the toxic effects of poisonous plants.

3.1.3. BSM naming of a plant

BSM practitioners name the medicinal plants mainly based on plant color and morphology. Quite often the given name of a plant is suffixed with the color of the flower such as *dkar-po* (white), *nag-po* (black), *dmar-po* (red), *gser-po* (yellow), *sngon-po* (blue) and *smug-po* (purple). For example, *Rhododendron anthopogon* D. Don is locally named as *dwa-li dkar-po* after its white flower (Fig. 2a), *Cavea tanguensis* Smith as *ming-can nag-po* after its dark flower (Fig. 2b), *Rhodiola crenulata* J. D. Hooker

& Thomson as *sro-lo dmar-pa* after its red flower (Fig. 2c), *Inula grandiflora* Willd. as *ming-can ser-po* after its yellow flower (Fig. 2d), *Meconopsis simplicifolia* Walpersas *utpal sngon-po* after its blue flower (Fig. 2e) and *Onosma hookeri* Clarke as *'bri-mog* after its purple flower and purple roots (Fig. 2f).

Plants are also named after the type of habitat. For example, *Polygonum macrophyllum* D. Don is locally named as *spang-ram* after its habitat type *spang* (meadows) and therefore it can be grouped under the origin *spang-sman* (medicinal plants growing in the meadows). Similarly, *Juniperus indica* Bertol grows in the habitat type *la* (often called *gangs-ri*) and is named as *la-shug*, and *Lepisorus contortus* Ching



Fig. 3. Medicinal plants named on the basis of their appearances and resemblances with human and animal body parts (courtesy: Phurpa Wangchuk collection): a) *D. Hatagirea* named as *dbang-po-lag-pa*, b) *E. arborescens* as *mkhal-ma-zho-sha-nag-po*, c) *P. flagellaris* as *glang-sna*, d) *P. longiflora* as *lug-ru*, e) *R. tricuspis* as *chu-rug-lbal-lag*, f) *D. drepanocentrum* as *bya-rkang*.

grows in the habitat type rocky cliffs and is thus named as *brag-spospabased* on their resemblances with the appearances of human and animal body parts (Fig. 3). For example, the rhizome of *Dactylorhizahatagirea* (D.Don) Soó resembles a human hand (*lag-pa*) and is thus named as *dbang-po lag-pa* (Fig. 3a). *Erythrina arborescens* Roxb. is named as *mkhal-ma zho-sha nag-po* after its kidney-shaped seed (*mkhal-rdog*) (Fig. 3b), *Pedicularis flagellaris* Benth. as *glang-ma* after its flower which resembles an elephant trunk (*glang-sna*) (Fig. 3c), *Pedicularis longiflora* D. Don as *lug-ru* after its flower which resembles a sheep horn (*lug:* sheep and *ru:* horn) (Fig. 3d), *Ranunculus tricuspis* Maxim. as *chu-rug lbal-lag* after its leaves which resembles a frog's webbed feet (*lbal-lag*) (Fig. 3e) and *Delphinium drepanocentrum* Munz as *bya-rkang* after its flower which resembles a bird's leg (*bya-gi-rkang-pa*) (Fig. 3f).

Knowledge of *Sowa Rigpa* medical names and the local vernacular names of the plants are valuable and our knowledge on both of these terminologies play critical roles in correct identification of the medicinal plants from our fieldwork in eastern Bhutan. Therefore, we have informally gathered information from our two local *Brokpa* guides about the local *Jop-kha* (vernacular names) of the 50 plant species and are presented in Table 1. They were also asked if their two communities would be interested to collect some potential medicinal plants for MSP. While these two guides said that the community would be very happy to take part in the government-run medicinal plants collection program including farmers training on sustainable collection of medicinal plants, future study to understand the perceptions and confirm the willingness of the communities is required.

Table 1

List of alpine medicinal plants identified from Merak and Sakteng regions.

Botanical Name, Family and Specimen number [29,30,31]	g.so-ba-rig-pa name	<i>Jop-kha</i> (local name)	Parts used	Altitude (masl)	Aspect	Growth form/ Habit	Conservation status IUCN/ Catalogue of life [33]
Aletris pauciflora (Klotzsch) HandMazz.	′dam-bu-ka-ra	tsa-lep-metog	Whole	4010	NE	Grass	††
Allium macranthum Baker	ri-sgog	lha-tshog-metog	Whole	4010	NW	Herb	††
(Amaryllidaceae, 5) Anaphalis contorta (D. Don) Hook. f.	spra-ba	tag-tse-metog	Whole	4010	SE	Herb	††
Androsace stigillosa Franchet	sga-tig-nag-po	pharam-metog	Aerial part	4010	NE	Herb	**
Bergenia purpurascens (Hook. f. & Thomson) Engl.	ga-dur	lhum-la-metog	Aerial part	4025	SW	Herb	**
(Saxifragaceae, NC) Boschniakia himalaica Hook. f. & Thomson (Orobranchaceae, 82)	stag-ma'i-yung-	dre-gog-pa	Seed, flower	4100	NW	Parasitic	††
Cassiope fastigiata (Wall.) D. Don	NA	au-lu	Aerial part	4080	NW	Shrub	††
Cremanthodium decaisnei C.B. Clarke	sha-la-yu-ring	NA	Flower	4090	SE	Herb	††
Cynoglossum wallichii G. Don (Boraginaceae, NC)	nad-ma-'byar-ma	roba-metog	Whole	3925	SE	Herb	††
Dactylorhiza hatagirea (D. Don) Soó	dbang-lag	puth-pa-metog	Root	4090	SE	Herb	††
Fragaria nubicola (Hook. f.) Lindl. ex Lacaita	bri-rta sa-'dzin	se-se-brep	Whole	3990	SE	Herb	††
Gentiana algida Pall.	spang-rgyan-	spang-rgyan-	Flower	4100	NW	Herb	**
Gentiana urnula Harry Sm.	gang-ga-chung	pang-sgon-metog	Whole	4010	SE	Herb	††
Geranium procurrens Yeo	ga-dur	NA	Root	3900	SE	Herb	**
Hypecoum leptocarpum Hook. f. & Thomson	par-pa-ta	langa-metog	Whole	4080	NW	Herb	††
Juniperus squamata Lamb.	shug-pa-tsher-	paam-shing	Leaves	4100	SW	Shrub	LC
Lilium nanum Klotzsch & Garcke	a-bi-kha	NA	Whole	4120	NW	Herb	**
Ligularia amplexicaulis DC.	NA	dole-chew-chew	Whole	3990	NW	Herb	**
Meconopsis horridula Hook. f. & Thomson	tsher-ngon	kuen-goen-	Whole	3965	NW	Herb	**
Meconopsis paniculata (D. Don) Prain	utpal-ser-po	chu-ser-metog	Whole	3990	SE	Herb	**
Megacodon stylophorus (C.B. Clarke) Harry	NA	Kiyoa	Whole	3900	SE	Herb	**
Morina betonicoides Benth.	spyang-tser-lo-	NA	Aerial part	4100	SW	Herb	††
Parnassia nubicola Wall ex Royle	dngul-tig	NA	Aerial part	3979	SE	Herb	††
Pedicularis megalantha D. Don	lug-ru-dmar-po	lug-ru	Whole	3959	SE	Herb	††
(Orobranchaceae, 72) Pedicularis siphonantha D. Don	dre-glang	lug-ru	Whole	3900	SE	Herb	††
(Orobranchaceae, 75) Pedicularis oliveriana Prain	lug-ru-smug-po	garam-metog	Flower	3900	SE	Herb	††
Phlomis rotata Benth. ex Hook. f.	rta-lpags	boram-metog	Whole	3979	SE	Herb	††
(Lamiaceae, NC) Phlomis younghusbandii Mukerjee	lug-mur	NA	Root	3990	SE	Herb	**
Pleurospermum hookeri C.B. Clarke	tang-kun-dkar-	khan-spos	Root	3965	NW	Herb	††
Polygonum macrophylla (D. Don)	po spang-ram	ray-mong-metog	Root	4010	NE	Herb	††
Potentilla arbuscula D. Don	sped-ma'-me-tog	kae-tse-metog	Flower	4010	SE	Shrub	††
Potentilla eriocarpa Wall. ex Lehm	NA	NA	Whole	4120	NW	Herb	††
(Rosaceae, 84) Potentilla fulgens Wall. ex Hook. f.	seng-ge-bar-ma	drolom-metog	Bark	3990	NW	Herb	††
(Rosaceae, NC) Potentilla peduncularis D. Don	NA	NA	Leaves, buds,	3990	SE	Herb	† †
(Rosaceae, NC) Primula sikkimensis Hook. f.	shang-dril-ser-po	NA	root Flower	3800	NW	Herb	††
(Primulaceae, 86) Ranunculus brotherusii Freyn (Ranunculaceae, 88)	lche-tsha	choekor-metog	Whole	4100	NW	Herb	††

(continued on next page)

Table 1 (continued)

Botanical Name, Family and Specimen number [29,30,31]	g. <i>so-ba-rig-pa</i> name	<i>Jop-kha</i> (local name)	Parts used	Altitude (masl)	Aspect	Growth form/ Habit	Conservation status IUCN/ Catalogue of life [33]
Rheum australe D. Don	chu-rtsa	kir-zhung-ma-	Root	3979	SE	Herb	† †
Rhododendron anthopogon D. Don (Ericaceae, 92)	ba-lu-dkar-po/ dwa-li metog	ba-lu	Flower	3959	NW	Shrub	††
Rhododendron setosum D. Don (Ericaceae, 93)	ba-lu-nag-po	ba-lu	Flower	3959	NW	Shrub	††
Rhododendron glaucophyllum Rehder (Ericaceae, 94)	stag-ma'i-lo-ma/ zhing	stag-shing	Leaves	4010	SE	Shrub	††
Rhodiola dumulosa (Franch.) S.H. Fu (Crassulaceae, NC)	tshad-chung-pa	NA	Whole	4000	NW	Herb	††
Rumex nepalensis Spreng. (Polygonaceae, NC)	sho-mang	sho-ma	Whole	3980	SE	Herb	††
Sabina squamata (BuchHam. Ex Don) Antoine	spa-ma	shug-tsher	Aerial parts	4010	SE	Shrub	††
(Cupressaceae, NC) Saxifraga melanocentra Franch. (Saxifragaceae, NC)	ʻod-ldan-dkar-po	NA	Whole	3900	SE	Herb	††
Saxifraga parnassifolia D. Don (Saxifragaceae, 104)	gser-tig	lamai-zhugthri	Whole	4010	NW	Herb	††
Spiraea canescens D. Don (Rosaceae, NC)	smag-shad- chung-ba	lang-ma	Whole	3979	SE	Shrub	**
Saussurea gossypiphora D. Don (Asteraceae, 100)	bya-rgod-sug-po	baa-poi	Whole	4100	NW	Herb	††
Taraxacum officinale F.H. Wigg. (Asteraceae, 111)	khur-mong	NA	Whole	3845	SE	Herb	††
Usnea sp. (Parmeliaceae, NC)	dngul-skud	She-li-dkar-po	Whole	3959	SE	Lichen	**
Viola biflora L. (Violaceae, NC)	rta-rmig	NA	Flower	3900	SE	Herb	††

NB: masl meters above sea level, NA Not Available, SE South-East, NW North-West, SW South-West, NE North-East.

†† – This taxon has not yet been assessed for the IUCN Red List but is in the catalogue of Life;

** - This taxon has not yet been assessed for the IUCN Red List, and also is not in the Catalogue of Life;

LC - Least Concern.

NC - Not Collected.

3.2. Botanical identification and distribution of medicinal plants in the Merak and Sakteng Gewogs of the Eastern Himalayas of Bhutan

Using BSM ethnotaxonomical and modern taxonomical concepts, a total of 50 medicinal plants (Table 1) were recorded from 13 different places along the altitudinal gradient of 3500 to 4120 masl in the alpine meadows of Merak and Sakteng. Of these, 26 medicinal plants were recorded between 3500–3999 masl and 24 medicinal plants above an altitude of 4000 masl. Out of 13 study plots surveyed, the highest number of medicinal plants recorded in the Samnyasa (3880–3979 masl) region was 19 medicinal species closely followed by Yanglay-yangchen (3800–4100 masl) with 18 medicinal plants, and Drangmazur (3900–3990 masl) with 13 species. Tshonag (3810–3959 masl) had the least number of medicinal plants with only two species recorded (Table 2).

These 50 medicinal plants belong to the habitat groups/life forms of herbs, shrubs, parasitic plants, grass and lichens. About 78% of the medicinal plants identified belong to the life form of herbs, which is distantly followed by shrubs with 16% and 2% each comprising parasitic plants, grass and lichens (Fig. 4). Representative photos of medicinal plants identified from Merak and Sakteng Gewogs are given in Fig. 5. Locally, the young shoots of *Meconopsis paniculata* and flowers of *Gentiana urnula* were extensively harvested by the yak herders and the people of two communities for consumption as vegetables. *Cordyceps sinensis* is also consumed by the *Brokpas* as health supplements by soaking them in their locally brewed wine for several days. *Hypecoum leptocarpum* is the only medicinal plant used locally in the form of a decoction as a home remedy against the common cough and cold.

3.3. Current utilisation of medicinal plants in BSM formularies

We analysed the current BSM formulations [34,35] for the number of species utilization, use frequency of each plant as an ingredient and types of diseases treated. Only 12 of the 50 medicinal species, which were identified from Merak and Sakteng Gewogs, are currently in use as ingredients in BSM (Table 3). These 12 plant species are: Fragaria nubicola, Rhododendron anthopogon, Juniperus squamata, Dactylorhiza hatagirea, Gentiana urnula, Hypecoum leptocarpum, Rheum australe, Gentiana algida, Meconopsis horridula, Pleurospermum hookeri, Geranium procurrens and Ligularia amplexicaulis (Table 3). They are included as ingredients in 48 different formularies to treat more than 50 ailments and disorders. Out of these, R. anthopogon and Geranium procurrens are used the most as ingredients in 8 formularies, followed by J. squamata (in seven formularies) and F. nubicola (in six formularies) each. Pleurospermum hookeri and Ligularia amplexicaulis are used in at least with one formulary each. Dactylorhiza hatagirea, listed as an IUCN Red-list species that is critically endangered [33], is also used as a major ingredient in polyingredient traditional medicine formulations.

These medicinal plants can be used in BSM as a whole plant, or as parts such as roots, aerial parts, leaves, flowers, seeds, mixed pats and bark. The Fig. 6 shows that 50% (25 species) of the medicinal plants which we have identified from Merak and Sakteng Gewogs are used as a whole plant in the BSM formulations. Top diseases treated by these BSM medicinal plants/formulations are wounds, burns, injuries, phlegm and bile disorders, cough and cold, fractured bones, swelling, and all types of fever (Fig. 7). For example, *Meconopsis horridula* is used for treating bone fracture, wounds and revitalizing the synovial fluids [36].

Table 2

Distribution pattern and availabilit	y status of 50 medicinal	plants in 13 study	areas from Merak	and Sakteng regions.
--------------------------------------	--------------------------	--------------------	------------------	----------------------

Botanical name	Medicinal plant species availability status in study plots												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Aletris pauciflora (Klotzsch) HandMazz.					••••								
Allium macranthum Baker													
Anaphalis contorta (D.Don) Hook. f.						••					•••		
Androsace stigillosa Franchet													
Bergenia purpurascens (Hook. f. & Thomson) Engl.													
Bistorta macrophylla (D.Don) Soják					•••	•••		•••	•••				
Boschniakia himalaica Hook. f. & Thomson													·
Cassiope fastigiata (Wall.) D.Don													
Cremanthodium decaisnei C.B. Clarke									•				
Cynoglossum wallichii G.Don													
Dactylorhiza hatagirea (D.Don) Soó													
Fragaria nubicola (Hook.f.) Lindl. ex Lacaita													
Gentiana algida Pall.													
Gentiana urnula Harry Sm.													
Geranium procurrens Yeo													
Hypecoum leptocarpum Hook. f. & Thomson													
Juniperus squamata Lamb.													
Liuum nanum Klotzsch & Garcke													
Liguiaria ampiexicautis DC.													
Meconopsis norridula Hook, I. & Inomson													
Meconopsis paniculata (D. Doli) Prain													
Megacodon stylophorus (C.B. Clarke) Harry Sill.													
Parnassia nubicola Wall av Poulo													
Padicularis megalantha D Don													
Pedicularis ineguantha D.Don													
Pedicularis siphonanina D.Don													
Phlomis rotate Benth, ex Hook f													
Phlomis vounghusbandii Mukeriee													
Pleurospermum hookeri C.B. Clarke													
Potentilla arbuscula D Don													
Potentilla eriocarpa Wall, ex Lehm													
Potentilla fulgens Wall, ex Hook, f.	•••												
Potentilla peduncularis D.Don													
Primula sikkimensis Hook. f.													
Ranunculus brotherusii Freyn													
Rheum australe D.Don						••	•						
Rhododendron anthopogon D.Don	•••										•••		
Rhododendron setosum D.Don					•••			•••	•••		•••	•••	
Rhododendron glaucophyllum Rehder													
Rhodiola dumulosa (Franch.) S.H. Fu													•
Rumex nepalensis Spreng.													
Sabina squamata (BuchHam. Ex Don) Antoine					·								
Saxifraga melanocentra Franch.		·											
Saxifraga parnassifolia D. Don	•												
Spiraea canescens D. Don						•							
Saussurea gossypiphora D.Don													•
Taraxacum officinale F.H.Wigg.							•						
Usnea sp.		•											
Viola biflora L.							·						

NB: 1 - Drangmazur, 2 - Nyakchungla, 3 - Tshonag, 4 - Dangla, 5 - Samnya, 6 - Kamloong, 7 - Sernyasa, 8 - Tshogara, 9 - Lumbur, 12 - Nagapa, 11 - Kargyap, 12 - Kayakpa, 13 - Yanglayyangchen; Medicinal plant species status was determined as per the method described inWangchuk et al., (2016a) [7] and represented as \cdots - Abundant, \cdots - Available, \cdot - Rare.

3.4. Phytochemical content of medicinal plants identified and their pharmacological activities

4. Discussion

The literature review on each of the 50 medicinal plant species, which were recorded from Merak and Sakteng, were performed to gather information on the phytochemical nature and pharmacological properties of each plant. Of 50 medicinal plants, we found 35 species have been previously studied for their phytochemical nature and pharmacological activities, and the remaining 15 medicinal plants had no published scientific literature (Table 4). It would be worthwhile to focus future scientific investigations on these unexplored medicinal plants.

Sowa Rigpa is practiced worldwide and it has its own pharmacopoeias and traditional medical textbooks such as 'Khrungs-dpe dri-med shel gyi me-long, Shel-gong shel-phreng, and Rgyud-bzhi [4,6,97,98]. These medical texts have unique concepts on classification and description of diseases and medicinal plants. In terms of ethnotaxonomical and plant identification framework, which was based on Tibetan Rgyud-bzhi and Shel-gong shel-phreng, Boesi [99,100] has previously described them in detail. Similar ethnobotanical framework focusing on Sowa Rigpa practiced by anchi of Dolpo in Nepal was reported by Lama et al. [101] and Ghimire et al. [102]. However, no such works were reported on Bhutanese Sowa Rigpa Medicine. Due to the local beliefs, culture, traditions and multi-lingual societies of Bhutan and the constant processes



Fig. 4. Growth forms of identified medicinal species.

of review, adaptation, innovation and evolution of BSM; the BSM practices are slightly different than the mainstream Tibetan tradition of *Sowa Rigpa* [2]. Such variations are to be expected, as the *materia medica* of mainstream Tibetan *Sowa Rigpa* should not be considered as standard and static both in time and space, but as a tradition that has been constantly evolving in several countries with its adaptations to

Table 3					
List of priority medicinal a	plant species	for the Menion	g Sorig I	Pharmaceut	icals

Rank	Use Frequency	Species Name
1	8	Rhododendron anthopogon
1	8	Geranium procurrens
2	7	Juniperus squamata
3	6	Fragaria nubicola
4	5	Hypecoum leptocarpum
5	3	Rheum australe
5	3	Meconopsis horridula
6	2	Dactylorhiza hatagirea
6	2	Gentiana urnula
6	2	Gentiana algida
7	1	Pleurospermum hookeri
7	1	Ligularia amplexicaulis

Use Frequency: number of frequency/times individual medicinal plant species used as ingredients in various traditional medicine formulations as per the *Monograph on Traditional Medicine of Bhutan* (2015) [31].

local vegetation, culture and foreign influences [100]. *Sowa Rigpa* has viably served as a healthcare modality in the region for centuries (even given differences in the plants that exist in different countries utilizing the tradition), and the present study explored the option of improving



Fig. 5. Medicinal plants representing five different life forms (courtesy: Karma Yeshi collection): a) *Aletris pauciflora,* b) *Morina betonicoide,* c) *Meconopsis paniculata,* g) *Meconopsis horridula* representing herbs; d) *Boschniakia himalaica* representing parasites; e) *Usnea* sp. representing fungi; f) *Rhododendron anthopogon* representing shrubs. H) Lake (Tsho-sngon-byung) near Samnya at altitude of 3979 masl where diverse medicinal herbs and shrubs grow nearby.

Table 4

Scientific status of medicinal	plants identified from	Merak and Saktens	g Gewogs and	their phytochemical/	pharmacological propertie
			, ,,		

Botanical Name	Traditional therapeutic indications [27.28.37.38.39.40.41.42.43.44.45.46.47]	Phytochemical/pharmacological properties (Literature review)
41		
Aletris pauciflora	Useful for treating pneumatic fever, liver and lung diseases.	Aqueous and methanol extracts of whole plant exhibited antioxidant activity [48]
Allium macranthum	Anti-inflammatory and useful for gastritis, tuberculosis and <i>rlung</i> (air) disorders.	Not studied Essential oil of flowers showed antiovidant activity [41]
Androsace stigillosa	Useful for edema, dropsy, fever and infections. Also assuage penis-plug syndrome.	Tested positive for tannins [49]; Methanolic extract exhibited
Ũ		strong anti-influenza viral activity [50]
Bergenia purpurascens	Used for relieving cough and reducing inflammation.	Methanolic extracts showed antimicrobial activity and
Bistorta macrophylla	Antidiarrheal antidysenteric and alleviates stomach pain	Methanolic extract showed antioxidant activity [43]
Boschniakia himalaica	Heals lung infections, tumour and blood disorders.	Compounds isolated from it exhibited feeding deterrent activity against <i>Tribolium</i> . <i>castaneum</i> adults [52]
Cassiope fastigiata	Incense in Merak and Sakteng; In Inida, leaves – making namkeen tea. Plant's smoke is used to ease respiration. Leafy twigs ground into a paste and applied on	Methanolic extract showed potent anti-herpes viral activity [50]
Cremanthodium decaisnei	burns. It is also ceremonial, ritual, and whole plant is used as incense. Abates severe bilious fever, headache, phlegm and bile disorders, and poisoning; suppress great pain of illnesses, and heals severe wounds and iniuries.	Not studied
Cynoglossum wallichii	Heals fractured bone, wound, and swelling.	Not studied
Dactylorhiza hatagirea*	Aphrodisiac, boost spermatogenesis and nourishes body.	Rhizomatous part showed resistance against all gram positive and gram negative bacteria [44]; Lyophilized aqueous extract of roots
		increases testosterone level in male rats [53]; clinical data on increasing the level of testosterone also enhance sexual desire and
		arousability in male adults [54]
Fragaria nubicola*	Anthelmintic and heals neurological and chest infections, and lung inflammation.	Fruit juice showed neuroprotective effects and antioxidant effects [55]
Gentiana algida*	Febrifuge and useful for sore throat.	Aqueous acetone extract of whole herb were active against the human pathogenic yeast <i>Candida albicans</i> [56]
Gentiana urnula*	Anti-diarrheal and detoxifier.	Flower extract showed excellent antioxidant activity [57]
Geranium procurrens*	Antidiarrheal, antitoxin and antimalarial. Useful for cough and cold, bronchitis and swelling of limbs.	Not studied
Hypecoum leptocarpum*	Useful for treating common cough and cold, skin diseases, blood pressure and poisonings.	Contains alkaloids [58]
Juniperus squamata*	Alleviates kidney inflammation and reduces accumulation of defective serous fluid in the joints.	Ethanol and methanol extracts showed broad spectrum of antibacterial activity [59]: contains sesquiterpenes [60]
Ligularia amplexicaulis*	Digestive and against emesis from indigestion, and in case of bile and phlegm diseases. Heals sores, dries serous fluid, and cures old epidemic diseases.	Essential oil from aerial parts showed antibacterial activity against Gram-negative bacteria [61]
Lilium nanum	Antidote, heals bone fracture and head injuries.	Not Studied
Meconopsis horridula* Meconopsis paniculata	Allays bone fracture, wounds and it is useful for revitalizing the synovial fluids. Useful for fever related to lung and liver disorders. Digestive and allays phlegm	Extract showed anti-tumour activity [36] Contains alkaloids [62,63]
Megacodon stylophorus	Used to clear heat and treat hepatopathy and gallbladder diseases.	Ethanol extract of whole plant showed anti-proliferative activity, cytotoxicity and antimicrobial activity [64]
Morina betonicoides	Emetics and purgatives used in cases of indigestion and phlegm disorders.	Not studied
Parnassia nubicola	Useful for bile disorders and ganglion related problems.	Methanol extract of whole plant showed antioxidant activity [65]
Pedicularis megalantha Pedicularis sinhonantha	Antidote and useful for infestinal disorders.	Not studied Four compounds isolated from whole plants [66]
Pedicularis oliveriana	Antidote, anti-inflammatory, antidiarrheal and febrifuge, they are used in treatment of poisoning, inflammation of stomach/intestines, diarrhoea and to	Not studied
	heal obstinate wounds and sores.	
Phlomis rotate	Accumulation of serous fluids in the bone, skin and wound, headache, fever, cough, worm infections and swelling caused by cold. Promotes blood circulation.	Contains iridoid glycosides [67]
Phlomis vounehushandii	Eliminates blood stasis. Anti-inflammatory. Kelieves pains Overcome or cure respiratory disorders, tuberculosis, swelling, lesions and cold	Isolated compounds showed anti-melanogenic activity [68]
Pleurospermum hookeri*	Antidote, anti-inflammatory and heals heart disorders.	Contains volatile compounds [69]
Potentilla arbuscula	Allays cough and cold.	Not Studied
Potentilla eriocarpa	Used in the form of tea in diarrhoea, kidney stones; arthritis and intestinal troubles.	Not studied
Potentilla fulgens	Alleviates cough and cold.	Compounds and crude methanol extract showed good anticariogenic potential and antibacterial activity [70]; Root
		extract showed high antitumor activity [71]; methanol extract of root showed hypoglycemic and anti-hyperglycemic activity [72]; root extract showed anti-hyperlipidemic activity [38]; chemical constituents from aerial parts and roots showed antioxidant
Potentilla peduncularis	Leaf-buds boiled in water is used for bathing to relieve fever. Influenza, cough and viral infections.	activity [/2,/3,/4,/5,/6,//,/8,/9] Not Studied
Primula sikkimensis	The paste of root is used to cure profuse menstruation. Febrifuge, antidiarrheal (used for children) and alleviates cardiovascular disorders.	Methanol extract showed thrombolytic activity [80]
Ranunculus brotherusii	Antiseptic, antipyretic, heals wound and dries pus.	Not studied
Rheum australe*	Antipyretic, improves digestion, and heals wound.	Root extracts showed anti-inflammatory activity [81]; Aqueous and methanol extracts of rhizome showed antibacterial, cytotoxicity and antioxidant capacity [39]
		(continued on next page)

(continued on next page)

Table 4 (continued)

Botanical Name	Traditional therapeutic indications [27,28,37,38,39,40,41,42,43,44,45,46,47]	Phytochemical/pharmacological properties (Literature review)
Rhododendron anthopogon*	Febrifuge for lung disorders and alleviates dropsy and other swelling caused by phlegm disorders (<i>ba-dkan-cha-bab</i>). Boost immune system.	Essential oil from aerial parts shoed weak anti-inflammatory activity, significant antibacterial activity and anticancer activity [82]
Rhododendron setosum	Allays <i>grang-ba</i> (including sexually transmitted infections) and <i>gang-lhog</i> (inflammation of the throat and muscle tissues). Used as incense for pacifying gods, demi-gods, deities and spirits.	Cold methanol extract showed antibacterial and antifungal activities [83]; methanol extract showed fair anti-cancerous activity [84]
Rhododendron glaucophyllum	Hemostatic and neutralizes other toxic side effects of medicine. Also used in incense products that pacifies gods, demi-gods, deities and spirits.	Not studied
Rhodiola dumulosa	Refresh lungs and abates fever due to pneumonia; useful for breathing problems; cure mouth diseases and improves speech.	Eight phenolic compounds isolated from the methanol extract of root [85]
Rumex nepalensis	Powder of leaves along with butter is used to treat scabies. Whole plant is boiled with water and the liquid is applied as wash for reducing body pain.	Hexane and methanol extracts showed significant antipyretic activity [86]; Root extract showed psychopharmacological activity in rats and mice [87]; Root extract showed antimicrobial activity [88,89]
Sabina squamata	Alleviates liver diseases and cracked skin; useful for piles and haemorrhoids.	Not studied
Saxifraga melanocentra	Protect from combined blood and bile diseases. Essence-extraction will enhance longevity, intelligence, appearance and strength. In Tibetan medicine, whole plant has quality to strengthen body and prolong life. It is useful to person with symptoms such as pale face and gums and general physical weakness which may relate to anaemia. It restores any imbalance of the three humours and improves the function of the senses. Also decrease the amount of impure blood in the body.	Ethanolic extract showed <i>in vitro</i> anti-HCV (Hepatitis C Virus) activity [90]
Saxifraga parnassifolia	Heals wounds and alleviates cough and cold, and bile disorders including jaundice.	Not studied
Spiraea canescens	Heals wounds, dries away puss; useful for skin diseases and indigestion.	Compounds isolated from the plant showed potent radical scavenging activity [91]
Saussurea gossypiphora	Analgesic and alleviates blood and liver disorders.	Contains flavonoids [92]
Taraxacum officinale	Removes toxin from the body (detoxifier). Allays fever arising from stomach disorders.	Showed anti-diabetic activity due to its anti-hyperglycemic, anti- oxidative, and anti-inflammatory properties [93]
Usnea sp.	Heals lung, liver, nerve and poison related diseases.	Ethanolic and methanolic extracts showed antibacterial activity [94]; ethanolic and methanolic extracts showed antioxidant activity [95]; Usnic acid showed potent antitermite activity [94]
Viola biflora	Diaphoretic, antipyretic, febrifuge, eczema, cancer and epilepsy.	Isolated compounds showed cytotoxicity [96]

NB: Species identified by an asterisk are priority species for Menjong Sorig Pharmaceuticals (MSP) which is used as ingredients in the current Bhutanese Sowa Rigpa Medicine/formulations.



Fig. 6. Plant parts used by plants and their relative frequency in medicinal formulations by part.

on existing traditional methods. This would eliminate any possible implication that traditional methods have been found to be inherently flawed.

We found that there is some relationship between the BSM ethnotaxonomical and the botanical (scientific) classification system at the following levels: *shing* correspond to the plant kingdom, *me-tog-can gyi shing* to the division of angiosperm, *me-tog med-pa'i shing* to the division of gymnosperm and *rigs* to species. Some levels of similarities are also seen in terms of morphological plant descriptions. However, many levels of botanical plant classification such as phylum, family and species, as well as many botanical nomenclatures, are not expressed in the BSM plant classification system. The plant names described in BSM were mainly drawn from their tastes, color, habitat and physical appearances and resemblances with other objects. Two specimens that are botanically-identified as distinct species can be identified as having the same properties and ethnotaxonomical identification in BSM. For example, it is likely that *Veronica ciliata* Fisch. and *V. himalensis* D. Don were both collected by the BSM practitioners under the same local name *ldum-nagdom-mkhris*, which is being used as a substitute for bear bile.

The BSM has served Bhutan for thousands of years and it is recognized today as critical cultural heritage. It also forms the basis of at least three of the four pillars of the Bhutan's unique developmental policy called Gross National Happiness (GNH). These pillars are: a) preservation of culture and traditions, b) preservation of environment, c) sustainable socio-economic development, and d) good governance. Preservation and promotion of BSM through scientific study is beneficial for patients' safety as well as for the job market. BSM adds value to plants by recognizing and cultivating their medicinal qualities. BSM also promotes recognition of their economic values, garnering respect for the preservation and sustainable utilisation of medicinal plants in the country. BSM engages rural farmers and nomads in the medicinal plants collection program and generates income for these farmers through collection of wild plant species and cultivation of high value medicinal plants [8].

With the above objectives in mind, especially for benefiting *Brokpas* of the two remote Gewogs (Merak and Sakteng) in northeastern Bhutan, we have conducted the first systematic medicinal plant survey in these regions sonce there is no reports or published literature from these regions so far. The current study botanically identified 50 medicinal plants growing at altitude ranges of 3880–3979 masl. These 50 plants were mostly herbaceous types (39 species in total) and belong to 25 families and 39 genera. Interestingly, the entire herb with roots intact is the most preferred ingredient in BSM [103] and consequently herbs constitute the maximum medicinal species collected by MSP for their BSM formulations [104,105]. Part of the reason for the preference of herbs in BSM could be that they are relatively easier to collect, dry, store and transport and also extract bioactive compounds from them. At



Fig. 7. Ailment categories and number of plants that have ethnotaxonomical indications for these ailments.

least 12 medicinal species (Table 3), which are currently in demand at MSP, are found growing abundantly in Merak and Sakteng Gewogs. Engaging *Brokpas* in their collection program would, not only generate income for them but could also benefit MSP in procuring medicinal plants sustainably domestically.

Additionally, these medicinal plants could be the potential sources of new drugs. Through literature review of the published studies, we found that 35 plant species (Table 4) identified in this study were studied for their phytochemical nature and pharmacological properties. For example, Corydalis dubia Prain [15,37,106-108], Pleurospermum amabile [37,107,109–111], Corydalis crispa [107,109,112], Corydalis calliantha [15,113], Aconitum laciniatum, A. orochryseum, Ajania nubigena, Codonopsis bhutanica, Meconopsis simplicifolia [15,107,111,114–118], Hypecoum leptocarpum, Podophyllum hexandrum, Lepisorus contortus [15], Thalictrum chelidonii, Erysimum bhutanicum, Aconogonon tortuosum, Thlaspi arvense, and Aletris pauciflora [119] have been studied both phytochemically and pharmacologically. The above studies not only provide a scientific basis for their medicinal uses in BSM but also led to the discovery of new drug compound leads: cheilanthifoline (Corydalis calliantha and C. dubia) and Coreximine (from C. crispa)- antimalarial lead compounds [112,113], isomyristicin and bergapten (from Pleurospermum amabile and C. crispa respectively) - anthelmintic drug leads [109], and capnoidine - new anti-inflammatory lead compound from C. dubia [15] just to name a few. Most importantly, from the content analysis of the published literature of medicinal plant species identified in the current study, we found that 15 species medicinal plants were never/least studied before, which are worthy of further scientific studies including phytochemical and pharmacological screening.

5. Conclusions

BSM has well-documented pharmacopoeia and traditional medical textbooks, which describe the unique ethnotaxonomical concepts, classification systems and descriptions of medicinal plants. From the medicinal plants survey in the Merak and Sakteng Gewogs in Bhutan, we have botanically identified 50 medicinal plants. Of these 50 species, 12 of them are currently in demand at MSP for formulating various polyingredient medicines. Collecting them from the study areas would economically benefit both the *Brokpa* communities and MSP. However, for the long-term sustainability of these medicinal plants, it would be important to train the *Brokpa* communities on sustainable collection and conservation methods and educate the policy makers about their conservation status and the ecological conditions. For example, *D. hatagirea* is one of the major ingredients used in the polyingredient BSM formulations. However, this species is listed as an IUCN Red-list species

that is critically endangered and a proper conservation and domestication strategy must be put in place before allowing the communities to collected this species from its wild natural habitat.

Of 50 medicinal plants identified from two Gewogs, 35 of them were scientifically studied for their phytochemical/pharmacological properties and the remaining 15 species remains unstudied. These unstudied species have future potential to uncover novel phytochemicals and biological properties, and also generate scientific data to support the traditional medical claims of BSM. To improve the quality, safety and efficacy of BSM, it is important to investigate both the individual medicinal plant and the BSM formulations for their pharmacological activities, safety and toxicities. For any resource-constraint (resources include research facilities, funding and expertise) country including Bhutan, conducting such detailed scientific investigations are unrealistic. Therefore, our compiled data on the known phytochemical and pharmacological studies of each plant (presented in this article) will be very handy and useful for the patient population, healthcare providers and policy-makers. This information will be also useful to guide future studies and analysis on the ethnopharmacology of BSM.

Acknowledgements

Authors would like to thank the Merak Gewog administration for their administrative support and study consent, and the people of Merak village for their hospitality, holistic collaboration and assistance in data and specimens collection. We also thank Mr. Samten (Senior Pharmacognosist) for his help in identification of the plants and Menjong Sorig Pharmaceuticals (MSP), Kawajangsa for giving access to their herbarium specimens.

References

- S. Craig, V. Adams, Global pharma in the land of snows: Tibetan medicines, SARS, and identity politics across nations, Asian Med. 4 (2008) 1–28.
- [2] P. Wangchuk, S.G. Pyne, P.A. Keller, An assessment of the Bhutanese traditional medicine for its ethnopharmacology, ethnobotany and ethnoquality: textual understanding and the current practices, J. Ethnopharmacol. 148 (1) (2013) 305–310.
- [3] K. Yeshi, T. Wangdi, N. Qusar, J. Nettles, S.R. Craig, M. Schrempf, P. Wangchuk, Geopharmaceuticals of Himalayan *Sowa Rigpa* medicine: ethnopharmacological uses, mineral diversity, chemical identification and current utilisation in Bhutan, J. Ethnopharmacol. 223 (2018) 99–112.
- [4] D.T. Phuntshok, Shel-Gong-Shel-phreng, T.M.A.I Publishers, India, 1994.
- [5] J. Dorje, 'jam dpal rdorje [of Mongolia]) [18. cent.?]. An Illustrated Tibeto-Mongolian Materia Medica of Ayurveda, Edited by Prof. Dr. Lokesh Chandra From the Collection of His Holiness Z.D. Gomboev With a Foreword by E. Gene Smith, International Academy of Indian Culture, New Delhi, 1971.
- [6] G. Dorji, 'Khrungs-dpe-dri-med-shel-gyi-me-long (Immaculate Crystal Mirror of Materia Medica Illustrations), Mi rigs dpe skrun khang, Beijing, 1995.
- [7] P. Wangchuk, D. Wangchuk, J.A. Hansen, Traditional Bhutanese medicine (g.so-

ba-rig-pa): an integrated part of the formal health care services, Southeast Asian J. Trop. Med. Public Health 38 (2007) 161–167.

- [8] P. Wangchuk, K. Namgay, K. Gayleg, Y. Dorji, Medicinal plants of Dagala region in Bhutan: their diversity, distribution, uses and economic potential, J. Ethnobiol. Ethnomed. 12 (28) (2016) 1–34.
- [9] P. Wangchuk, S.G. Pyne, P.A. Keller, Ethnobotanical authentication and identification of khrog-sman (lower Elevation Medicinal Plants) of Bhutan, J. Ethnopharmacol. 134 (2011) 813–823.
- [10] P. Wangchuk, Tashi, Quality assurance of the university medical education, hospital services and traditional pharmaceutical products of the Bhutanese *So-wa-rig*pa health care system, BMC Complement. Altern. Med. 16 (2016) 283.
- [11] Department of Agriculture (DoA), Data From Meteriological Section, Ministry of Agriculture and Forests, Thimphu, 1988.
- [12] Bhutan Media Studies (BMS), At the Heart of the Kingdom: Profile of 205 Gups and Gewogs of Bhutan, (2013).
- [13] The ISE Code of Ethics, International Society of Ethnobiology, (2006) http:// ethnobiology.net/code-of-ethics/.
- [14] Access and Benefit Sharing (ABS) Policy of Bhutan, (2015) http://www.nbc.gov. bt/wp-content/uploads/2010/06/ABS-Policy-of-Bhutan-2015.pdf.
- [15] K. Yeshi, P. Yangdon, S. Kashyap, P. Wangchuk, Antioxidant activity and the polyphenolic and flavonoid contents of five high altitude medicinal plants used in Bhutanese Sowa Rigpa Medicine, J. Biol, Act. Prod. Nat. 7 (1) (2017) 18–26.
- [16] A.J.C. Gierson, D.G. Long, Flora of Bhutan: Including Records of Plants From Sikkim, Vol. 1 Part 1. Edinburgh: Royal Botanic Garden, (1983).
- [17] A.J.C. Gierson, D.G. Long, Flora of Bhutan: Including Records of Plants From Sikkim, Vol. 1 Part 2. Edinburgh: Royal Botanic Garden, (1984).
- [18] A.J.C. Gierson, D.G. Long, Flora of Bhutan: Including Records of Plants From Sikkim, Vol. 1 Part 3. Edinburgh: Royal Botanic Garden, (1987).
- [19] A.J.C. Gierson, D.G. Long, Flora of Bhutan: Including Records of Plants From Sikkim, Vol. 2 Part 1. Edinburgh: Royal Botanic Garden, (1991).
- [20] A.J.C. Gierson, D.G. Long, Flora of Bhutan: Including Records of Plants From Sikkim, Vol. 2 Part 2. Edinburgh: Royal Botanic Garden, (1999).
- [21] A.J.C. Gierson, D.G. Long, Flora of Bhutan: Including Records of Plants From Sikkim, Vol. 1 Part 3. Edinburgh: Royal Botanic Garden, (2001).
- [22] H.B. Naithani, Flowering Plants of India, Nepal and Bhutan: Not Recorded in Sir JD Hooker's Flora of British India, Surya Publications, Dehradun, 1990.
- [23] H.J. Noltie, Flora of Bhutan: Including a Record of Plants From Sikkim Darjeeling, Vol.3 Part 1. Edinburgh: Royal Botanic Garden, (1994).
- [24] H.J. Noltie, Flora of Bhutan, vol.3 Part 2. Edinburgh: Royal Botanic Garden, (2000).
- [25] G. Pandey, Medicinal Plants of Himalaya, Sri Satguru Publications, Indological and Oriental Publishers, Delhi, 2000.
- [26] O. Polunin, A. Stainton, Flowers of the Himalaya, Oxford University Press, Oxford, 1997.
- [27] P. Wangchuk, P.A. Keller, S.G. Pyne, M. Taweechotipatr, A. Tonsomboon, R. Rattanajak, S. Kamchonwongpaisan, Evaluation of an ethnopharamacologically selected Bhutanese medicinal plants for their major classes of phytochemicals and biological activities, J. Ethnopharmacol. 137 (2011) 730–742.
- [28] P. Wangchuk, Ugyen Samten, J. Thinley, S.H. Afaq, High altitude plants used in Bhutanese traditional medicine (g.so-ba-rig-pa), Ethnobot 20 (2008).
- [29] The Plant List. Version 1.1, (2013) http://theplantlist.org/.
- [30] eFloras.org. 2008. [http://www.efloras.org].
- [31] Tropicos.org, Missouri Botanical Garden, (2016) http://ww.tropicos.org.
- [32] K. Sabernig, On the history of the murals in the medical college of Labrang, Asian Med. 7.2 (2012) 358–383.
- [33] IUCN, The IUCN Red List of Threatened Species. Version 2017-3, (2017) (accessed 10 March 2018), http://www.iucnredlist.org.
- [34] Formulary of Traditional Medicine of Bhutan (FTMB), Ministry of Social Service, Thimphu, Bhutan, 1983.
- [35] Monograph on Traditional Medicine of Bhutan (MTMB), Ministry of Health, Thimphu, Bhutan, 2015.
- [36] J. Fan, Y. Wang, X. Wang, P. Wang, W. Tang, W. Yuan, L. Kong, Q. Liu, The antitumour activity of *Meconopsis horridula* Hook, a traditional Tibetan medicinal plant, in Murine Leukemia L1210 Cells, Cell. Physiol. Biochem. 37 (2015) 1055–1065.
- [37] C. Shepherd, P. Giacomin, S. Navarro, C. Miller, A. Loukas, P. Wangchuk, A medicinal plant compound, capnoidine, prevents the onset of inflammation in a mouse model of colitis, J. Ethnopharmacol. 211 (2017) 17–28.
- [38] D. Syiem, P.Z. Khup, A.B. Syiem, Effects of *Potentilla fulgens* Linn. On carbohydrate and lipid profiles in diabetic mice, Pharmacologyonline 2 (2009) 787–795.
- [39] R.K. Gupta, G.B. Bajracharya, R.N. Jha, Antibacterial activity, cytotoxicity, antioxidant capacity and phytochemicals of *Rheum austral* rhizomes of Nepal, J. Pharmacogn. Phytochem. 2 (2014) 125–128.
- [40] T. Li, T. Wang, Chemical constituents of volatile oil of *Pleurospermum hookeri*, Zhongcaoyao 32 (2014) 780–781.
- [41] K. Rawat, K. Prasad, G. Bisht, Phytochemical analysis and antioxidant activity essential oil of *Anaphalis contorta* from Uttrakhand Himalayas, Anal. Pharm. Res. 6 (2017) 00172.
- [42] C. Kletter, M. Kriechbaum, Tibetan Medicinal Plants, Medpharm GmbH Scientific Publishers, Germany, 2001.
- [43] B. Liu, M. Wang, X. Wang, Phytochemical analysis and antibacterial activity of methanolic extract of *Bergenia purpurascens* against common respiratory infection causing bacterial species in vitro and in neonatal rats, Microb. Pathog. 117 (2018) 315–319.
- [44] S. Ranpal, An Assessment of Status and Antibacterial Properties of Dactylorhiza hatagirea in Annapurna Conservation Area (A Case Study of Paplekharka, Lete

VDC, Mustang), B.Sc Forestry Research Thesis submitted to Tribhuvan University, Institute of Forestry, Pokhara, Nepal, 2009.

- [45] N.P. Manandhar, Some less known medicinal plants of Rasuwa district (Nepal), Int. J. Crude Drug Res. 18 (1980) 147–151.
- [46] N.P. Manandhar, A survey of medicinal plants of Jajarkot district, Nepal, J. Ethnopharmacol. 48 (1995) 1–6.
- [47] M. Tomczyk, K.P. Latté, Potentilla-a review of its phytochemical and pharmacological profile, J. Ethnopharmacol. 122 (2009) 184–204.
- [48] P. Wangchuk, P.A. Keller, S.G. Pyne, A.C. Willis, S. Kamchonwongpaisan, Antimalarial alkaloids from a Bhutanese traditional medicinal plant *Corydalis dubia*, J. Ethnopharmacol. 143 (1) (2012) 310–313.
- [49] P. Wangchuk, Phytochemical Analysis, Bioassays and the Identification of Drug Lead Compounds From Seven Bhutanese Medicinal Plants. Doctor of Philosophy Thesis, School of Chemistry, University of Wollongong, 2014.
- [50] M. Rajbhandari, R. Mentel, P.K. Jha, R.P. Chaudhary, S. Bhattarai, M.B. Gewalia, N. Karmacharya, M. Hipper, U. Lindequist, Antiviral activity of some plants used in Nepalese Traditional medicine, Evid.-Based Complementary Altern. Med. 6 (2007) 517–522.
- [51] S. Chandra, S. Saklani, A.P. Mishra, R.K. Agrawal, *In vitro* antioxidant activity and phytochemical screening of Garhwal Himalaya medicinal plants, Int. J. Med. Res. Health Sci. 5 (8) (2016) 35–43.
- [52] J. Cao, Z.L. Liu, S.S. Du, Z.W. Deng, Feeding deterrents from the tubers of Boschniakia himalaica against the red flour bettle, *Tribolium castaneum*, J. Med. Plants Res. 6 (18) (2012) 3506–3511.
- [53] M. Thakur, V.K. Dixit, Aphrodiasic activity of *Dactylorhiza hatagirea* (D.Don) Soo in male albino rats, Evid.-Based complement, Altern. Med. 4 (2007) 29–31.
- [54] J. Bancroft, The endocrinology of sexual arousal, J. Endocrinol. 186 (2005) 411–427.
- [55] P.B. Rakhunde, S.A. Ali, Antioxidant and cytoprotective effect of *Fragaria nubicola* on ischemia reperfusion induced brain injury, Annals Experiment. Biol. 2 (2014) 33–38.
- [56] R.X. Tan, J.L. Wolfender, W.G. Ma, L.X. Zhang, K. Hostettmann, Secoiridoids and antifungal aromatic acids from *Gentiana algida*, Phytochem. 41 (1996) 111–116.
- [57] K. Kusakari, T. Fukuhara, A. Motoyama, N. Ochiai, T. Watanabe, Y. Sugimoto, The corrected structure of depressoside, an antioxidative iridoid glucoside extracted from the flowers of *Gentiana urnula* Harry Sm, Nat. Prod. Res. 30 (2015) 954–959.
- [58] G.L. Zhang, G. Rücker, E. Breitmaier, R. Mayer, Alkaloids from Hypecoum leptocarpum, Phytochem. 40 (2000) 1813–1816.
- [59] S.C. Sati, P. Kumar, Assessment of Himalayan Juniper, Juniperus squamata Buch-Ham ex. D. Don for phytochemical screening and antimicrobial potential against some infection causing pathogens, World J. Pharm. Res. 4 (2015) 998–1011.
- [60] Y.H. Kuo, I.C. Yang, C.S. Chen, Y.T. Lin, Five new sesquiterpenes from the heartwood of *Juniperus squamata* Lamb, J. Chin, Chem. Soc. 34 (1987).
- [61] D. Joshi, M. Nailwai, L. Mohan, A.B. Melkani, *Ligularia amplexicaulis* (wall.) DC. Essential oil composition and antibacterial activity, J. Essent. Oil Res. (2018), https://doi.org/10.1080/10412905.2018.1427636.
- [62] A. Brossi, The alkaloids: Chemistry and Pharmacology, National Institute of Health, Bethesda, Maryland, 1986 (Eds).
- [63] R.H.F. Manske, The alkaloids: Chemistry and Physiology, UniRoyal Limited Research Laboratory, Canada, 1968 (Eds).
- [64] C. Liu, Z. Liao, S. Liu, L. Ji, H. Sun, Two new 2,3-Seco-Hopane triterpene derivatives from *Megacodon stylophorus* and their antiproliferative and antimicrobial activities, Planta Med. 80 (2014) 936–941.
- [65] N.Rao Sudhanshu, S. Mittal, E. Menghani, Evaluation of anti-malarial and antioxidant effect of *Parnassia nubicola* methanolic extract, Int. J. Curr. Pharm. Res. 4 (3) (2012) 77–79.
- [66] J.Q. Yang, W.J. He, N.H. Tan, H.B. Chu, W.L. Zhang, H.F.Dai Mei, Chemical constituents of *Pedicularis cephalantha* Franch and *P. Siphonantha* Don, Nat. Pro. Res. Dev. 21 (2009) 600–603.
- [67] C.-Z. Zhang, C. Li, S.-L. Feng, J.-G. Shi, Iridoid glycosides from Phlomis rotata, Phytochem. 30 (1991) 4156–4158.
- [68] M. Li, C. Zhang, L. Wei, P. Faan, Q. Zhang, Z. Jia, Determination of five iridoid glycosides in *Phlomis younghusbandii* by HPLC, Zhongguo Zhong Yao Za Zhi 3695 (2011) 594–597.
- [69] T. Li, T. Wang, Chemical constituents of volatile oil of Pleurospermum hookeri, Zhongcaoyao. 32 (2001) 780–781.
- [70] A. Choudhary, U. Bihade, A.K. Mittal, A. Chatterjee, U.C. Banerjee, I.P. Singh, Anticariogenic potential of *Potentilla fulgens* extract and its chemical constituents, IJOP. 9 (2017) 83–91.
- [71] D. Syiem, C. Syngai, B. Kharbuli, H. Kayang, B.S. Khongswir, Anti-tumor activity of crude root extract of Potentilla fulgens, Indian J. Drugs Dermatol. 40 (2003) 124–125.
- [72] D. Syiem, G. Syngai, P.Z. Khup, B.S. Khongwir, B. Kharbuli, H. Kayang, Hypoglycemic effects of *Potentilla fulgens* L. In normal and alloxan-induced diabetic mice, J. Ethnopharmacol. 83 (1-2) (2002) 55–61.
- [73] M.A. Bos, B. Vennet, M.T. Meunier, M.P. Pouget, A. Pourrat, J. Fialip, Procyanidins from tormentil: antioxidant properties towards lipoperoxidation and anti-elastase activity, Biol. Pharm. Bull. 19 (1996) 146–148.
- [74] Y.H. Choi, M.J. Kim, H.S. Lee, C. Hu, S.S. Kwak, Antixoidant compounds in aerial parts of Potentilla fragarioides, Korean J. Pharmacogn. 29 (1998) 79–85.
- [75] I. Gurbuz, A.M. Ozkan, E. Yesilada, O. Kutsal, Antiulcerogenic activity of some plants used in the traditional medicine of Bulgaria and Italy, J. Ethnopharmacol. 101 (2005) 313–318.
- [76] M. Leporatti, S. Ivancheva, Preliminary comparative analysis of medicinal plants used in the traditional medicine of Bulgaria and Italy, J. Ethnopharmacol. 87 (2003) 123–142.

- [77] G. Miliuskas, T.A. Vanbeek, P.R. Venskutonis, J.P.H. Linssen, P.D. Waard, E.J.R. Sudholter, Antioxidant activity of *Potentilla fruticosa*, J. Sci. Food Agric. 84 (2004) 1997–2009.
- [78] M. Sambyal, A. Dogra, S. Koul, A. Ahuja, Rapid *in-vitro* propagation of *Potentilla fulgens* Wall. a Himalayan alpine herb of medicinal value, J. Plant Biochem. Bio. 15 (2006) 143–146.
- [79] H. Tunon, C. Olavsdotter, L. Bohlin, Evaluation of anti-inflammatory activity of some Swedish medicinal plants. Inhibition of prostaglandin biosynthesis and DAFinduced exocytosis, J. Ethnopharmacol. 48 (1995) 61–76.
- [80] Md. Jakaria, Md. Islam, Md.S. Islam, M.B. Talukder, C.D. Clinton, M. Ibrahim, Thrombolysis potential of methanol extracts from the five medicinal plants leaf, available in Bangladesh, Pharmacologia. 8 (2017) 78–82.
- [81] N.S. Chauhan, B.S. Kaith, S.K. Mann, Anti-inflammatory activity of *Rheum australe* roots, Int. J. Pharmacogn. 30 (2008) 93–96.
- [82] G. Innocent, S. Dall'Acqua, G. Scialino, E. Banfi, S. Sosa, K. Gurung, M. Barbera, M. Carrara, Chemical composition and biological properties of *Rhododendron an*thopogon essential oil, Molecules. 15 (2010) 2326–2338.
- [83] J. Lamichhane, S.B. Chettri, M. Bhandari, S. Pokhrel, A. Pokharel, J.K. Sohng, Ethnopharmacological survey, phytochemical screening and antibacterial activity measurements of high altitude medicinal plants of Nepal: a bioprospecting approach, IJTK. 13 (2014) 496–507.
- [84] J. Lamichhane, S.B. Chhetri, M. Bhandari, S. Pokhrel, R. Timilsina, T.M. Shrestha, Antiproliferative bioassay of extremophilic medicinal plants from Langtang Himalayan range of Nepal, J. Biomolecule Reconstruction. 9 (2012) 180–185.
- [85] Q. Liu, Z.L. Liu, X. Tian, Phenolic components from Rhodiola dumulosa, China J. Chinese material medica. 33 (4) (2008) 411–413.
- [86] S. Venkatesh, M.R. Bommineni, R.D. Reddy, M. Ramesh, Antipyretic activity of *Rumex nepalensis* roots, Niger, J. Nat. Prod. Med. 7 (2003) 53–54.
- [87] L. Ghosh, G. Arunachalam, T. Murugesan, M. Pal, B.P. Saha, Studies on the psychopharmacological activities of *Rumex nepalensis* Spreng. Root extract in rats and mice, Phytomed. 9 (2002) 202–206.
- [88] H.-X. Liang, H.-Q. Dai, H.-A. Fu, X.-P. Dong, A.-H. Adebayo, L.-X. Zhang, Y.-X. Cheng, Bioactive compounds from *Rumex* plants, Phytochem. Lett. 3 (2010) 181–184.
- [89] S. Yadav, S. Kumar, P. Jain, R.K. Pundir, S. Jadon, A. Sharma, Antimicrobial activity of different extracts of roots of *Rumex nepalensis* Spreng, Indian J. Nat. Prod. Resour. 2 (2011) 65–69.
- [90] G.Y. Zuo, Z.Q. Li, L.R. Chen, X.J. Xu, *In vitro* anti-HCV activities of *Saxifraga melanocentra* and its related polyphenolic compounds, Antivir. Chem. Chemother. 16 (2005) 393–398.
- [91] M.I. Choudhary, N. Naheed, A. Abbaskhan, S. Ali, Au. Rahman, Hemiterpene glycosides and other constituents from Spiraea canescens, Phytochem. 70 (2009) 1467–1473.
- [92] S. Zheng, H. Chen, X. Shen, Study on flavonoids from Saussurea gossypiphora D. Don. Xibei Shifan daxue xuebao, Ziran Kexueban. 4 (1990) 42–45.
- [93] K. Schütz, R. Caele, A. Schieber, Taraxacum a review on its phytochemical and pharmacological profile, J. Ethnopharmacol. 107 (2006) 313–323.
- [94] S. Kathirgamanathar, D.E. Williams, R.J. Andersen, K. Bombuwela, D.D. Silva, V. Karunaratne, B-Orcinol depsidones from the lichen *Usneas*p. From Sri Lanka, Nat. Prod. Res. 19 (2006) 695–701.
- [95] B.C. Sharma, S. Kalikotay, Screening of antioxidant activity of lichens Parmotrema reticulatum and Usnea sp. From Darjeeling hills, India, Iosr J. Pharm. 2 (2012) 54–60.
- [96] A. Herrmann, R. Burman, J.S. Mylne, G. Karlsson, J. Gullbo, D.J. Craik, R.J. Clark, U. Göransson, The alpine violet, *Viola biflora*, is a rich source of cyclotides with potent cytotoxicity, Phytochem. 69 (2008) 939–952.
- [97] Y.Y. Gonpo, The Subsequent Tantra From the Secret Quintessential Instructions on the Eight Branches of the Ambrosia essence Tantra, Men-Tsee-Khang Publications, Dharamsala (Reprinted), 2011.
- [98] Y.Y. Gonpo, The Root Tantra and the Explanatory From the Secret Quintessential Instructions on the Eight Branches of the Ambrosia essence Tantra, 2nd ed., Men-Tsee-Khang Publications, Dharamsala (Reprinted), 2011.
- [99] A. Boesi, Plant knowledge among tibetan populations, in: A. Boesi, F. Cardi (Eds.), Wildlife and Plants in Traditional and Modern Tibet: Conceptions, Exploitation and Conservation, 33 Memoriedella Societa Italiana di scienze Naturali e del Museo Civico di storia Naturale di Milano, 2005, pp. 33–48.

- [100] A. Boesi, Plant categories and types in Tibetan materia medica, Tibet J. 31 (2006) 67–92.
- [101] Y.C. Lama, S.K. Ghimire, Y.A. Thomas, Medicinal Plants of Dolpo: Amchis Knowledge and Conservation.WWF Nepal Program, Kathmandu, Nepal, (2001).
- [102] S.K. Ghimire, D.B. Parajuli, T.N. Gurung, Y.C. Lama, Conservation of Plant Resources, Community Development and Training in Applied Ethnobotany at Shey-Phoksundo National Park and Its Buffer-zone, Dolpa. WWF Nepal program Report Series No. 38. WWF Nepal program, Kathmandu, Nepal, (1999).
- [103] P.D. Moore, Trials in bad taste. Nature. 370, 410-411.Naithani, H.B., 1990, Flowering Plants of India, Nepal & Bhutan (not Included in Sir J.D Hooker's Flora of British India), A Survey Publication, Debra Dun, India, 1994.
- [104] F.G. Coe, G.J. Anderson, Ethnobotany of the garifuna of Eastern Nicaragua, Econ. Bot. 50 (1996) 71–107.
- [105] P.M. Shrestha, S.S. Dhillion, Medicinal plant diversity and use in the highlands of Dolakha district, Nepal, J. Ethnopharmacol. 86 (2003) 81–96.
- [106] P. Wangchuk, T. Sastraruji, M. Taweechotipatr, P.A. Keller, S.G. Pyne, Anti-inflammatory, anti-bacterial and anti-acetylcholinesterase activities of two isoquinoline alkaloids-scoulerine and cheilanthifoline, Nat. Prod. Commun. 11 (12) (2016) 1801–1804.
- [107] P. Wangchuk, P.A. Keller, S.G. Pyne, M. Taweechotipatr, S. Kamchonwongpaisan, GC/GC-MS analysis, isolation and identification of bioactive essential oil components from the Bhutanese medicinal plant, Pleurospermum amabile, Nat. Prod. Commun. 8 (9) (2013) 1305–1308.
- [108] P. Wangchuk, P.A. Keller, S.G. Pyne, A.C. Willis, S. Kamchonwongpaisan, Antimalarial alkaloids from a Bhutanese traditional medicinal plant Corydalis dubia, J. Ethnopharmacol. 143 (1) (2012) 310–313.
- [109] P. Wangchuk, P.R. Giacomin, M.S. Pearson, M.J. Smout, A. Loukas, Identification of lead chemotherapeutic agents from medicinal plants against blood flukes and whipworms, Sci. Rep. 6 (2016) 32101.
- [110] P. Wangchuk, S.G. Pyne, P.A. Keller, M. Taweechotipatr, S. Kamchonwongpaisan, Phenylpropanoids and furanocoumarins as antibacterial and antimalarial constituents of the Bhutanese medicinal plant, Pleurospermum amabile, Nat. Prod. Commun. 9 (2014) 957–960.
- [111] P. Wangchuk, P.A. Keller, S.G. Pyne, M. Taweechotipatr, Inhibition of TNF- α production in LPS-activated THP-1 monocytic cells by the crude extracts of seven Bhutanese medicinal plants, J. Ethnopharmacol. 148 (2013) 1013–1017.
- [112] P. Wangchuk, P.A. Keller, S.G. Pyne, T. Sastraruji, M. Taweechotipatr, R. Rattanajak, A. Tonsomboon, S. Kamchonwongpaisan, Phytochemical and biological activity studies of the Bhutanese medicinal plant Corydalis crispa, Nat. Prod. Commun. 7 (5) (2012) 575–580.
- [113] P. Wangchuk, J.B. Bremner, R. Samten, S. Rattanjak, Kamchonwongpaisan, Antiplasmodial agents from the Bhutanese medicinal plant *Corydalis calliantha*, Phytother. Res. 24 (2009) 481–485.
- [114] P. Wangchuk, Samten, GC-FID Coupled GC-MS Analysis of the Essential Oil and the Recorded Biological Activities of *Meconopsis simplicifolia*, J. Biol. Act. Prod. Nat. 5 (6) (2016) 36–372.
- [115] P. Wangchuk, M.S. Pearson, P.R. Giacomin, L. Becker, J. Sotillo, D. Pickering, M.J. Smout, A. Loukas, Compounds derived from the Bhutanese daisy, *Ajania nubigena*, demonstrate dual anthelmintic activity against *Schistosoma mansoni* and *Trichuris muris*, PLoS Negl. Trop. Dis. 10 (2016) e0004908.
- [116] P. Wangchuk, S. Navarro, C. Shepherd, P.A. Keller, S.G. Pyne, A. Loukas, Diterpenoid alkaloids of *Aconitum laciniatum* and mitigation of inflammation by 14-O-acetylneoline in a murine model of ulcerative colitis, Sci. Rep. 5 (2015) 12845.
- [117] P. Wangchuk, P.A. Keller, S.G. Pyne, J. Korth, M. Samten, R. Taweechotipatr, S. Rattanajak, Kamchonwongpaisan, Antimicrobial, antimalarial and cytotoxicity activities of constituents of a Bhutanese variety of *Ajania nubigena*, Nat. Prod. Commun. 8 (2013) 733–736.
- [118] P. Wangchuk, J.B. Bremner, B.W. Samten, A.H. Skelton, R. White, S. Rattanajak, Kamchonwongpaisan, antiplasmodial activity of atisinium chloride from the Bhutanese medicinal plant, *Aconitum orochryseum*, J. Ethnopharmacol. 130 (2010) 559–562.
- [119] K. Yeshi, P. Yangdon, S. Kashyap, P. Wangchuk, Taxonomical identification of Himalayan edible medicinal plants in Bhutan and the investigation of selected plants for their phenolic contents and antioxidant activity, J. Biol. Act. Prod. Nat. 7 (2) (2017) 89–106.