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Development of quality control parameters for two Bhutanese medicinal plants (*Aster flaccidus* Bunge and *Aster diplostephioides* (DC.) Benth. ex C.B.Clarke) using traditional and modern pharmacognostical platforms

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ABSTRACT

Bhutan's scholarly traditional medical system is called Bhutanese *Sowa Rigpa* medicine (BSM). It was integrated with the modern healthcare system in 1967. Over 200 medicinal plants are used to produce more than 100 poly-ingredient medicinal formulations. Although BSM is supported by well-documented principles, pharmacopoeias, diagnostic procedures, treatment regimens, and traditional quality assurance systems, modern quality control parameters have become essential to distinguish closely related species and prevent contamination from exogenous impurities. This study aims to establish reliable analytical methods and quality control parameters for *Aster flaccidus* Bunge and *Aster diplostephioides* (DC.) Benth. ex C.B. Clarke used as ingredients in the BMS poly-ingredient medicinal formulations. Furthermore, their reported phytochemicals and biological activities are also discussed in this study. Standard pharmacognostic techniques, including macroscopical and microscopical examinations of crude drugs, were employed to establish the quality control parameters for two *Aster* species. The physicochemical limits were determined as per the World Health Organization (WHO)-recommended guidelines and methods described in the Thai herbal pharmacopoeia. A high-performance thin-layer liquid chromatography (HPTLC) was used to develop a comparative chromatogram/phytochemical fingerprint for the crude extracts obtained from two *Aster* species. A literature review was conducted to record their isolated phytochemicals and biological activities. Two *Aster* species possess macro- and microscopic features such as colour, appearance, and shape. Physicochemical analysis of crude drugs from two *Aster* species including HPTLC fingerprinting of their methanol crude extracts also yielded adequate data to differentiate and confirm two *Aster* species before adding them to the

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BSM poly-ingredient medicinal formulations. From the literature review, only *A. flaccidus* was found to be studied for its phytochemical constituents, whereby 11 pure compounds were isolated from aerial parts and roots. The current study revealed distinct species-specific distinguishing features, including ecological adaptation, micromorphology, anatomy, physicochemical values, HPTLC chromatograms. These parameters can be used to authenticate the species identity and prevent adulterations, thereby improving the quality and safety of BSM formulations.

1. Introduction

Medicinal plants play a crucial role as principal components in many traditional and indigenous medicine systems worldwide, providing primary health services to approximately 80 % of the global population [1,2]. In Bhutan, over 200 medicinal plants are used as main ingredients to produce more than 100 poly-ingredient medicinal formulations in their traditional medicine system [3]. This system is widely known as *Sowa Rigpa* (Wylie transliteration is gso-ba rig-pa), which means "science of healing" [4]. This scholarly traditional medical system belongs to the larger corpus of Tibetan medicine and has been diligently passed down through canonical texts and oral teachings [3].

Tibetan medicine is not confined to Tibet and Bhutan. It is practiced worldwide, including in the Himalayan regions of India (Ladakh, Sikkim, and Himachal Pradesh), Nepal, Mongolia, Russia, and more recently, in European and North American countries like Switzerland, Germany, Canada, the United States of America (USA), and Mexico [5]. For many reasons, the so called Tibetan medicine in the West is known as *Sowa Rigpa* or *Nangpai Sman*, or Indigenous medicine or Bhutanese traditional medicine (BTM) in Bhutan. It is said that the Bhutanese physicians, known as Drungtshos, have adapted the Tibetan scholarly medical system to meet the local disease trends, local availability of medicinal plants, and the health care needs of the Bhutanese people [4]. Bhutan has hotter climates that would have influenced i) the lush growth of low-altitude medicinal plants and ii) disease prevalence (e.g., malaria, which is not heard in Tibet due to the colder climate). The BTM was integrated into the modern healthcare system in 1967. Since then, its services have been expanded to all hospitals and basic health centres nationwide. Today, more than 70 traditional medical centres in the country provide primary health care services to the people through the same hospital infrastructures. As a result, to provide quality and safe herbal medicines, there is an urgency to develop quality control parameters for both the medicinal raw ingredients and the formulated products.

Well-documented principles, pharmacopoeias, diagnostic procedures, and treatment regimens support this BTM system. For example, *che-pai-yen-lag-b.duen* (seven quality attributes of medicinal practices) provides a detailed description of traditional ways of monitoring the quality of BTM, especially medicinal plants [6]. Medicinal plant collection follows strict ethnoquality doctrines, including correct identification, right habitat and harvesting time, correct processing and detoxification methods, proper storage, and spiritual empowerment. Such factors may impact phytoconstituents/secondary metabolites of the plants, ultimately affecting the drug's therapeutic efficacy. Seasonal variations cause changes in the plant's chemical composition/secondary metabolites and, thus, their bioactivity [7,8]. For instance, in the medicinal herb *Lippia alba* (Mill.) N.E.Br. ex Britton & P.Wilson, phenylpropanoids were predominant during winter, and flavonoids were predominant during summer [9]. Therefore, scientific studies are essential to enhance herbal drugs' quality, safety, and efficacy. Moreover, it will foster mutual understanding, respect, and increased collaboration between traditional medicine and biomedicine [6]. The demand for herbal medicines is rising, driven by their perceived minimal or no side effects. However, this growing demand has also increased the risk of adulteration with incorrect or low-grade materials [10]. Thus, to ensure the safety of traditional medicines, accurately identifying their primary ingredients is the first critical step. One of the common sources of misidentification is using the same vernacular name for two or more entirely different species or the same genus.

As per the World Health Organization's (WHO) mandate for standardization and quality control of herbal drugs, the physico-chemical evaluation of crude drugs, including the selection and handling of raw materials, is considered essential [11]. As a result, much attention is often given to quality indices, such as macroscopic and microscopic examination, total ash values, moisture content, extractive values, crude fiber, qualitative and quantitative chemical evaluation, and chromatographic study [12,13]. Once the plant is dried and processed into powder form, it loses its morphological identity, becoming more susceptible to adulteration. It is possible to characterize crude powder microscopically; however, this technique will be difficult. Thus, using pharmacognostic parameters to authenticate the starting material becomes a critical step in the quality check of medicinal plants [14]. In the present study, we conducted comparative pharmacognostic studies between two closely resembling *Aster* species (*Aster flaccidus* Bunge and *A. diplostephioides* (DC.) Benth. ex C.B.Clarke), which are also easily mistaken for other species of the Asteraceae family. These species are essential ingredients in eight poly-ingredient formulations in BSM.

The *Aster* genus represents a group of perennial flowering plants that belong to the Asteraceae family and are commonly found in the temperate zone, particularly in North America [15]. With approximately 292 known species [16], some are prized for their ornamental value, while others have traditional uses in healing coughs due to their antibacterial and antiviral properties [17–19]. In Bhutan, *A. flaccidus* and *A. diplostephioides*, are recognised for their healing properties in conditions such as fractured cranium, dropsy, and as an anodyne for body aches affecting the upper part of the body. The BTM system uses formulations comprising these two species and other ingredients against at least 15 diseases and illnesses. Establishing pharmacognostic specifications for the medicinal plants used in these formulations is paramount to ensure the safety and quality of BTM healthcare services. The determination of standard quality control parameters through this study will aid in authenticating these two medicinal plants. Furthermore, we have comprehensively reviewed the existing literature on the phytochemical and biological properties of the two *Aster* species, which would

facilitate the development of molecular fingerprints, quality control parameters, and plant monographs. This study aims to facilitate the authentication and differentiation of the two *Aster* species, which would ultimately help improve the quality, safety, and efficacy of the BSM.

2. Materials and methods

The method used in this study includes collecting plant materials, assessing their ethnopharmacological uses, and a comparative anatomical and pharmacognostic evaluation of crude drugs from two *Aster* species. Furthermore, a phytochemical fingerprint for two *Aster* species was also generated using a high-performance thin-layer chromatography (HPTLC) technique.

2.1. Collection and preparation of plant materials

The aerial parts and stems of *A. flaccidus* and *A. diplostephioides* were collected from Lingshi under Thimphu district, Bhutan, 4000–4900 m above sea level (masl) between July and September. The plants were identified by referring to the Flora of Bhutan [20] and further authenticated by Mr. Samten (senior pharmacognosist at Mejong Sorig Pharmaceutical Corporation Limited, MSPCL). The herbarium specimens of *A. flaccidus* and *A. diplostephioides* were deposited at MSPCL with their voucher number 65 and 56, respectively. Fresh plant materials were rinsed several times under the running tap water and shade-dried. Dried plant materials were ground into fine powder to study their pharmacognostic characteristics. All *Sowa Rigpa* terms related to the two medicinal plants studied are described with a Wylie transliteration system.

2.2. Ethnopharmacological uses and macroscopic features of the plants

Ethnopharmacological uses of two *Aster* species included in this study were derived from various traditional scholarly texts, including *Shel-gong Shel-phreng* [21] and plant monographs [22] and the Handbook of Quality Control Practice [1] available in the Faculty of Traditional Medicine (FoTM) under the Khesar Gyalpo University of Medical Sciences of Bhutan (KGUMSB) at Kawajangsa, Thimphu. Biogeographical distribution, ecological adaptation, collection season, and botanical characteristics of two species, including dimensions, shape, and color texture, were recorded during the time of collection, and data were validated by referring to the Flora of Bhutan and other available literature [23,24]. The traditional formulations comprising these two species and diseases treated were described by consulting experts at FoTM and by referring to the published literature [6,25–27]. All data were presented in a tabular form, and the pictorial representation of the plants and their respective crude drugs were provided to support the data further.

2.3. Pharmacognosy of two Aster species

2.3.1. Plant anatomy of two Aster species using microscopy technique

Transverse sections (T.S.) of the stem from the fresh plant materials were prepared using a plant micro-technique [28,29] and observed and photographed using novex microscope K-range (Holland). A distinct cellular characteristic of each *A. flaccidus* and *A. diplostephioides*, observable under the microscope, was identified and described in detail.

2.3.2. Morphology of powdered crude drugs of two Aster species using microscopy

Powdered crude drugs were mixed well in BSM to prepare various formulations. The cellular features of two *Aster* species, including tracheids, vessels, fibres, and stomata, were previously reported in the medicinal plant monographs [22,30]. Following the same protocol, the microscopic characteristics of crude drugs of two *Aster* species were reproduced using a high magnification novex microscope K-range (Holland).

2.3.3. Physicochemical parameters of two Aster species

Physicochemical properties such as foreign matter, moisture content, total-ash value, acid-insoluble ash, alcohol soluble extractive values, and loss on drying for the crude drugs of two *Aster* species were calculated as per the Thai Herbal Pharmacopoeia [31], Bhutanese medicinal plant monographs [22,30,32] and the World Health Organization Monographs on Selected Medicinal Plants [33]. For each parameter, analysis was performed in triplicates.

2.3.3.1. Foreign matter. Plant raw materials should not contain contaminants/adulterants, including adulterations with cheap plant materials and other animal hair/faecal contamination, but $\leq$2 % are deemed acceptable [31,34,35]. The crude drugs of two *Aster* species were visually examined to determine the acceptable limits for the foreign matter content. Dried plant materials (100 g, in pieces of 1–5 cm) were spread on a clean sheet of paper and examined for foreign particles with the unaided eye and then with a hand-held magnifying glass lens. The percentage of foreign matters present in the crude drug was calculated as below:

$$\%\ foreign\ matter = 100 \times (a - b) \div a$$

where, 'a' and 'b' are the weights of crude drug before and after sorting, respectively.

2.3.3.2. Moisture content. As per the WHO quality control guidelines [36], an azeotropic distillation method was used to determine the

moisture content in the crude drugs of two *Aster* species. Briefly, a crude powder drug (2 g) was transferred to a clean round bottom flask, and 300 mL of toluene was added. The mixture was boiled for 2 h and then cooled at room temperature, separating the water and toluene layers. After the separation, water volume was recorded, and the percentage of moisture content was determined as per the methods described in Thai Herbal Pharmacopoeia [31,34]:

$$\%\ moisture\ content = (100 \times n) \div p$$

where, 'p' is the weight (g) of the substance to be examined, and 'n' is the net volume of water (in mL) obtained.

2.3.3.3. Loss on drying. The values for loss on drying were determined following the methods described in the Thai Herbal Pharmacopoeias [31,34]. First, a clean and sterile crucible was heated by placing it in an oven (110 °C) and weighing the crucible after cooling. This process was repeated until the weight became constant. Then, the powdered material (2 g) was heated in the crucible (110 °C) for 4 h. Three consecutive readings were taken for each sample until their constant weights were obtained. The percentage of loss on drying was calculated from the average weight of three independent experiments using the formula below:

$$\%\ loss\ on\ drying = 100 \times (n\ - n') \div n$$

where, n and n′ are the weights of crude powdered drug before and after drying, respectively.

2.3.3.4. Total ash value. Initially, the weight of the crucible was normalised through repeated measurement by placing it in the furnace at 600 °C. Once the weight of the crucible became constant, the powdered plant material (2 g) was placed in the crucible, heating it to the set temperature for 5 h. The sample was then left to cool in a desiccator. The sample was re-heated, and the weight was measured repeatedly until the constant weight of the ash was obtained. The average weight of the three independent measurements was used to calculate the percentage of the ash value using the formula below [31,34,35]:

$$\%\ ash\ value = 100 \times (n - n') \div n$$

where, n and n′ are the weights of crude powdered drug before and after incineration, respectively.

2.3.3.5. Acid-insoluble ash value. The sample's total ash was placed in a crucible, dissolved in hydrochloric acid (4 %, 4 mL HCl in 96 mL water), and filtered through an ashless filter paper, repeatedly rinsing with hot water until the filtrate was pH neutral. The residue and filter paper were placed in a crucible and incinerated at 600 °C until it turned ash completely. The crucible was cooled inside a desiccator, and the weight was recorded. It was re-heated until the constant weight was obtained, and the acid-insoluble ash percentage was calculated using the formula [31,34,35]:

$\%\ acid\ insoluble\ ash = (100 \times n') \div n$where, n = weight of the crude sample before heating, and n′ = weight of the acid insoluble ash.

2.3.3.6. Cold extraction for determining alcohol soluble extractive values. The dried crude drug (4 g) was coarsely powdered/ground, placed in a conical flask with alcohol (100 mL, triplicate), and macerated (6 h) with vigorous shaking (using an electric shaker). It was left at room temperature for 18 h and was filtered. The filtrate (25 mL) was transferred to a pre-weighed Petri dish using a volumetric pipette and then evaporated in a water bath (80 °C). It was cooled in a desiccator, weighed, and replaced in a water bath until a constant weight was obtained. The alcohol-soluble extractives were calculated as follows:

$$\%\ alcohol\ (ethanol) soluble\ extractive\ values = (100 \times a) \div b$$

where a = weight of the extract, b = weight of the powder.

2.4. HPTLC instrumentation, chromatographic method, and sample preparation

The HPTLC sample preparation and profile development was conducted following the methods described by Wangchuk and Jamtsho [37–39]. The instrumentation system (CAMAG®, Muttenz, Switzerland) consisted of a DigiStore2 digital system along with winCATS software ver.1.4.3 (CAMAG®, Muttenz, Switzerland), automatic developing chamber CAMAG ADC2, a Linomat 5 sample applicator with 100 μL syringes, and twin trough chambers (Analtech, USA). Thin layer chromatography (TLC) (aluminium sheets pre-coated with silica gel 60 F_{254}, Merck KGaA) glass plates (20 cm × 10 cm) were used for loading the samples and analysis. The powdered material of two *Aster* species (1 g) was mixed with methanol (10 mL AR grade) and kept in a water bath for 30 min at 50 − 60 °C to obtain its crude dry extract. Methanol was used to solubilise the dry extracts of the two crude drug samples (aerial parts of *A. diplostephioides* and whole plant of *A. flaccidus*), which were then applied to a pre-coated silica gel plate with the help of a micro-pipette. For the chromatographic analysis, crude drug extracts of both *Aster* species were spotted onto a single TLC plate (20 cm × 10 cm, glass) at two dilutions (5 μL and 10 μL) using CAMAG Automatic TLC Sample IV (ATS4). The plate was then developed at room temperature (21 °C) using a solvent system of methanol: chloroform (1:9) in a twin trough chamber (CAMAG), which was saturated for 20 min, and subsequently, the plate was allowed to run for approximately 40 min. The plate was scanned at the UV wavelengths of 254 nm and 366 nm using a CAMAG Visualizer: 201416, and the retention factor (Rf) of the crude drug was calculated

using the formula:

$$Retention\ factor\ (Rf)\ value = a \div b$$

where, a = distance travelled by the compound from the baseline, b = distance travelled by the solvent from the spotting baseline.

2.5. A literature review on the phytochemical and biological properties of two Aster species

A detailed literature review was conducted on the phytochemical constituents and their reported biological activities by mining available literature using relevant search keywords, including "*A. flaccidus*," "*A.diplostephioides*," "phytochemistry," "phytoconstituents," "isolation," bioactivity," and "analysis," in various databases such as Google Scholar, PubMed, SciFinder, and MEDLINE. Only a few studies have been reported on the phytochemical analysis of two *Aster* species, and the findings are reported in the result section.

2.6. Limitations of the method

An integrated traditional and modern pharmacognostical method was applied to develop preliminary quality control parameters for two medicinal *Aster* species. However, we could not determine the phytochemical composition and bioactivity of parts used as ingredients in BTM. The current study restricted the anatomical study to only parts of the plants used as ingredients. Analyzing other parts of the plants, for instance, the T.S. of rhizome could also have been interesting features to distinguish two *Aster* species. Moreover, the current BTM's protocol for processing fresh plant materials involves washing with tap water to remove soil and other debris, which could add moisture and facilitate fungal growth if they are nod dried completely. Therefore, similar future studies must include analyzing the crude drugs for fungal and heavy metal contaminations, which the current study could not achieve.

3. Results and discussions

3.1. Ethnobotanical characterization and traditional medicine formulations of two Aster species

In BTM, single plants are rarely used in treating diseases. The medicines contain 2–100 ingredients that are mixed using modern pharmaceutical equipment. In general, individual plant species are chopped, dried, and powdered and then mixed with other ingredients and made into different dosage forms such as tablets, pills, capsules, powders, syrups, ointments, and decoctions. For example, *A. flaccidus* and *A. diplostephioides* are dried, powdered, and then mixed with other ingredients to make three forms of essential traditional medicine drugs (ETMDs): *a-gar-35* (**1,** contains *A. diplostephioides* with 34 other ingredients, total 35 ingredients**)**, *thang-chen-25* (**2,** contains *A. flaccidus* with 24 other ingredients, thus, total 25 ingredients), and *rin-chen-byur-d.mar-25* (**3,** contains both *Aster* species with 23 other ingredients) (Table 1). Their usage in BSM is guided by ethnopharmacological descriptions in traditional *Sowa Rigpa* texts, for instance, *Shel-gong Shel-phreng* [21].

Aster flaccidus is known locally as "*lug-mig*" (Fig. 1A). The stem and flowers of *A. flaccidus* are used to prepare crude drugs. The ray florets are mauve, and the stem appears green when fresh but turns yellowish when dried (Fig. 1B). The leaves are also present in small quantities. The dried plant is used as an herbal ingredient in preparing three ETDMs (see **1, 2,** and **3** in Table 1). For example, *A. flaccidus*, along with 24 herbal ingredients, is used to prepare a herbal product called *thang-chen-25* (**2**), a capsule-based formulation. The recommended dosage is four capsules taken orally with lukewarm water during morning hours. It is prescribed for remedying many conditions such as stomach problems, cough and cold, fever, loss of appetite, febrifuge, poisoning (antidote), and chronic bronchitis. Likewise, a-*gar-35* (**1**) comprises thirty-five herbal ingredients, including *A. flaccidus*. This polyingredient herbal product is recommended for chronic fever, dry cough, arthritis, heart diseases, insomnia, epidemic fever, wandering pain, dehydration, and other

Table 1
Aster flaccidus Bunge (*lug-mig*) and *Aster diplostephioides* (DC.) C.B.Clarke (*a-byag-g.zer-'joms*) used in formulating three types of ETMDs.

S. No	ETMDs	Number of ingredients	Medication form	Route of administration	Therapeutic indications in BSM [40,42]	Precautions
1	a-gar-35 [a]	35	Tablet (500 mg)	Orally, three tablets during the evening, accompanied by hot water	Dry cough, chronic fever, cardiovascular disorder, arthritis, insomnia, pain, dehydration, and other ailments, such as blood and wind related disorders (*khrag-rlung*)	Contains aconite. Avoid meat and alcohol while taking this medication
2	thang-chen-25 [b]	25	Capsule (400 mg)	Orally, four capsules in the morning with warm water	Stomach disorder, loss of appetite, febrifuge, and poisoning	Store in a sealed container to shield from sunlight and moisture
3	rin-chen-byur-d. mar-25 [c]	25	Pill (500 mg)	Orally, one pill in the evening with hot water after it has cooled slightly	Neurological disorder, giddiness, fainting, stiff neck, and chronic headache	

[a] only *Aster diplostephioide used* as ingredients.
[b] only *Aster flaccidus* used as ingredients.
[c] both *Aster* species are used as ingredients; ETMDs – essential traditional medicine drugs; BTM – Bhutanese *Sowa Rigpa* medicine.

Fig. 1. Macroscopic characteristics: A. *Aster flaccidus* in natural habitat; B. *Aster flaccidus* crude drug; C. *Aster diplostephioides* in natural habitat; D. *Aster diplostephioides* crude drug.

diseases due to blood and wind disorders (*khrag-rlung*). The recommended dosage is three tablets to be taken in the evening with hot water [40,41].

Aster diplostephioides is locally known as *'a-byag-g.zer-'joms'*. The crude drug derived from *A. diplostephioides* is characterized by fragile flowers, odourless and tasteless hairy leaves, obscurely three-toothed ray florets, and lanceolate leafy bracts (Fig. 1C and D). It is an ingredient in two ETDMs (**1**, *a-gar-35,* and **3**, *rin-chen-byur-d.mar-25*). These two formulations are indicated for treating various ailments such as cardiovascular and neurological disorders, fever, giddiness, fainting, neck stiffness, brain diseases, heaviness in the head, and chronic headaches. The recommended dosage involves orally taking three tablets (for *a-gar-35*) and one pill (for *rin-chen-byur-d.mar-25*) in the evening with hot water after slight cooling (Table 1). *Rin-chen-byur-d.mar-25* formulation contains aconite as one

of the ingredients, and aconite is known for its toxicity; thus, avoiding meat and alcohol consumption is essential during this medication [42]. People of Manang district in central Nepal also consume dried flower of *A. diplostephioides*, in powder form (usually half spoonful) with a cup of boiled water to heal coughs and colds, headaches and sore throat, snake bites and scorpion sting, wounds, chest backbone pain, pulse pain (*nadhidhukhunue* in Nepali language) and numbness of limbs until recovery [43].

3.2. Botanical description and ecological distribution of two Aster species

The genus *Aster* belongs to the Compositae family. This genus predominantly inhabits the temperate zone and includes 500 species spread across the continent. Bhutan is home to 16 species [23,44]. Among the 16 species, only *A. flaccidus* and *A. diplostephioides* are ingredients for the BSM [40,42]. Morphological characteristics of two *Aster* species were recorded during the sample collection and cross-checked with the Flora of Bhutan and other available literature [23,24]. A comparative morphological description of the two species is given in Table 2 below. *Aster flaccidus* is an herbaceous erect perennial, growing up to 3–30 cm tall with long and slender rhizomes ensuring better adaptation in the subalpine or subarctic biome region. It has characteristic solitary flower heads, numerous mauves, linear, spreading ray florets, and oblong, long-pointed, woolly-haired involucral bracts. Leaves are oblanceolate, clustered at the rootstock, and stems are short with lanceolate clasping leaves and bristly hairy fruits [23]. The plant is native to several countries, including Bhutan, India, Nepal, Afghanistan, Kazakhstan, Pakistan, Uzbekistan, Mongolia, and Russia [45,46]. It prefers humid alpine heaths and subalpine meadows, groves, screes, and forests [23,24]. In Bhutan, it is distributed across Thimphu (Lingzhi), Paro (Upper Paro Chu district-Gafoo La), Bumthang (Marlung), and Gasa districts. It predominantly occupies open sandy slopes, meadows, screes, and shrubs within an altitude range of 3750–5025 masl [23].

Aster diplostephioides is also a herbaceous perennial plant. It grows in alpine savannas, damp alpine pastures, scrubland, marshy areas, riverine, floodplains, and coniferous or mixed forests within the altitude range of 2700–4600 masl [24,47]. *Aster diplostephioides* has distinctive taxonomist features such as numerous long, narrow, somewhat reflexed lilac ray florets. Disk florets start blackish and turn orange, while the ray florets are obscurely 3-toothed. Further, leaves are oblanceolate to linear-lanceolate, while the stem is leafy and shaggy-haired, and the fruit is complemented by a silky reddish pappus [23]. This plant is reportedly native to Bhutan, India, Nepal, Pakistan, and China. In Bhutan, it is usually found growing in Bumthang, Gasa, Haa, and Punakha districts at altitudes ranging from 3500 to 4200 masl [23].

Table 2
Comparative botanical characteristics of the two *Aster* species.

S. no	Parameters	*Aster flaccidus*	*Aster diplostephioides*
1	Plant height	Erect perennial, 3−30 (−40) cm tall	Perennial, 13−57 cm tall
	Rhizome	Long and slender	Robust and branched
2	Stem types	Stems 5−15 cm, erect, simple, whitish pubescent and sometimes glandular, white villous, more densely upward, sparsely to densely minutely stipitate-glandular	15−40 cm, erect, simple, sparsely to moderately villous, sparsely to densely minutely stipitate-glandular and pubescent above, or sometimes scapiform
3	Leaf types	Basal leaves spathulate or oblanceolate, 1.7–6.5 × 0.8–1.5 cm, acute or obtuse, base attenuate to acute and shortly petiolate, pubescent on both surfaces; cauline leaves sessile, oblong, or oblong-lanceolate,1.5–9 × 0.2–1.5 cm, sparse to moderate villous and minutely stipitate-glandular characters, margin entire or slightly serrate, ciliate, 3-veined	Basal leaves lanceolate or oblanceolate, 6–13 × 1.3–2 cm, acute, attenuate at base, entire, sometimes denticulate, glandular, or lightly pubescent; cauline leaves oblong or linear 1.3–15 × 0.8–2.3 (−4) cm, sparse to moderate villous, and minutely stipitate-glandular, margin slightly serrate to serrulate or entire, midvein prominent
4	Petiole length	Short or long (up to 7 cm)	Narrowly winged (up to 10 cm)
	Capitulum	Terminal, solitary, 3.5–5 cm in diameter	Terminal, solitary, 6–9 cm in diameter
	Involucre	Hemispheric, 1.2–1.7 cm diameter, 2−3 seriate	Hemispheric, 1.7–2.2 cm diameter, ±2 seriate
5	Phyllaries	Lanceolate, 0.7–1.3 × 0.1–0.2 cm, abaxially basally densely white lanate to sparsely white villous, minutely stipitate-glandular, margin scarious, sometimes purple-tinged, ciliate, membranous, multiveined, apex acuminate, often densely villous	Linear-lanceolate, 0.9–1.6 cm in length and 0.1–0.15 cm in width, apex acuminate, abaxial surface is lightly or moderately villous, blackish glandular with white hair at the base, margin scarious, with multiple veins
6	Ray florets	31–67 flower, corolla tube 0.1–0.2 cm, mauve or blue, lamina linear-lanceolate, 0.9–2.5 × 0.1–0.25 cm, abaxial hairy or glabrous, and glandular	45–93 flower, corolla tube 0.15 cm, mauve to purple or lilac-blue, lamina linear, 0.18–0.25 × 0.1–0.2 cm, glabrous, and glandular
7	Disk floret	Orange to yellow, 0.045–0.07 cm, limb funnelform, 0.35–0.5 cm, proximally lightly pubescent, lobes erect, triangular, 0.07–0.15 mm	Corolla is orange to yellow, 0.5–0.6 cm, apex blackish or brownish purple, tube, proximal limb pubescent and funnelform, 0.35–0.4 cm, lobes erect and triangular, ca. 0.1 cm
8	Achenes	Brownish, narrowly obovoid, 0.25–0.35 cm, sparsely strigillose, sparsely to moderately pubescent, rarely glabrous, glandular, 2-ribbed, ribs pronounced	Achenes obovoid, 0.3–0.35 × 0.1–0.15 cm, glandular and whitish pubescent, 4–6 ribbed
9	Pappus	Double, whitish, inner series 7 mm, outer series 2 mm, subpaleaceous	Double, whitish, inner series 5 mm, outer series 1–1.5 mm, subpaleaceous
10	Inflorescence type	Capitulum solitary	Capitulum solitary

Botanical characteristics described here were recorded during the sample collection and validated by referring to available literature [23,24].

3.3. Macroscopic and microscopic features of two Aster species

The macroscopic and microscopic analyses were conducted following the plant microtechnique [48], using a novex microscope K-range (Holland). This approach highlights the prominence of integrating a microscopy method in pharmacognosy to validate medicinal herbs [49]. The morphology of the crude drug and its distinct anatomical features under the microscope are given in Fig. 2.

A. flaccidus: Under low magnification (× 10), the stem's transverse section (T.S.) exhibited a hexagonal or octagonal shape with distinct ridges on the surface where the number of ridges determines the stem's shape. The epidermis was a single layer of rectangular cells, some of which formed pyramid-like outgrowths. The cortex zone consisted of parenchymatous cells of various shapes and sizes. The cortex comprised 4–5 layers of cells, while 8–10 layers were present at the ridges. The section consisted of 12–14 well-developed vascular bundles, with the ones at the ridges larger than those between them. Each vascular bundle was composed of well-defined zones of phloem and xylem. Towards the epidermis, a sclerenchymatous fibrous layer was connected to the xylem parenchyma. The vessels were relatively small, and the pith consisted of parenchymatous cells of various sizes, forming a polygonal shape surrounded by vascular bundles (Fig. 2A).

The crude drug of *A. diplostephioides* mainly consisted of short and small pieces of stem, flowers, and leaves without a noticeable odour or taste. The leaves and flowers were delicate, and the rootstock appeared blackish. Some parts of the fruit with yellow inside were also present. The stems were long and hairy at the base. The T.S. of the *A. diplostephioides* stems consisted of a circular shape with a single layer of parenchymatous cells without intercellular spaces. However, only part of the T.S. is shown in Fig. 2B. The peripheral region of epidermal cells consisted of a cuticle layer and multicellular hairs (trichomes). Beneath the epidermis, the cortex comprised 3–5 layers of parenchyma cells (Fig. 2B). The vascular bundles were in a ring shape, showing conjoint, collateral, and open characteristics, with the xylem towards the center and the phloem towards the periphery. The distinct cambium layer was absent, but the vascular bundle comprised sieve elements, fibers, and parenchyma in the phloem region. Toward the center was the pith region composed of thin-walled, rounded, or polygonal parenchymatous cells with minimal intercellular space. Additionally, certain portions of the pith region between vascular bundles comprised thin-walled, outwardly elongated parenchymatous cells known as medullary rays. The isolated elements from crude drug mainly consisted of fibres, tracheids, and vessels (Fig. 3A–E).

3.4. Physicochemical properties of two Aster species

Monitoring the quality control practices involving the starting materials to detect adulteration or mishandling of drugs is a crucial physiochemical parameter to ensure the reproducible quality of herbal products [37,50]. The physicochemical analysis of two *Aster* species involves assessing the foreign matter, total ash, acid-insoluble, alcohol-soluble extractives, and loss on drying. These parameters are vital for ensuring the quality control of herbal drugs in Bhutan (Table 3). A high ash value may indicate potential issues with drug preparation or the presence of foreign materials, inorganic salts, or silica as adulterants or substitutes in the crude drug sample. On the other hand, alcohol-soluble extractive indicates the solubility of the crude drug in the water [38,51]. A lower extractive value also signifies the addition of exhausted material, adulteration, or incorrect processing during drying, storage, or formulation [52]. The alcohol-soluble ash value of *A. diplostephioides* was 21 %, indicating higher solubility than *A. flaccidus*, which had a value of 7 %. However, the extractive value for both *Aster* species falls within the acceptable limit [38,53–55]. The MSPCL has established acceptable limits for total ash and acid insoluble values at 10 % and 8 %, respectively. Both *Aster* species had total and acid-insoluble ash (Table 3) values within these limits, indicating compliance with the accepted criteria [53–55]. The determination of ash values is necessary as it assesses the presence of silica, particularly in the form of sand and siliceous earth [56].

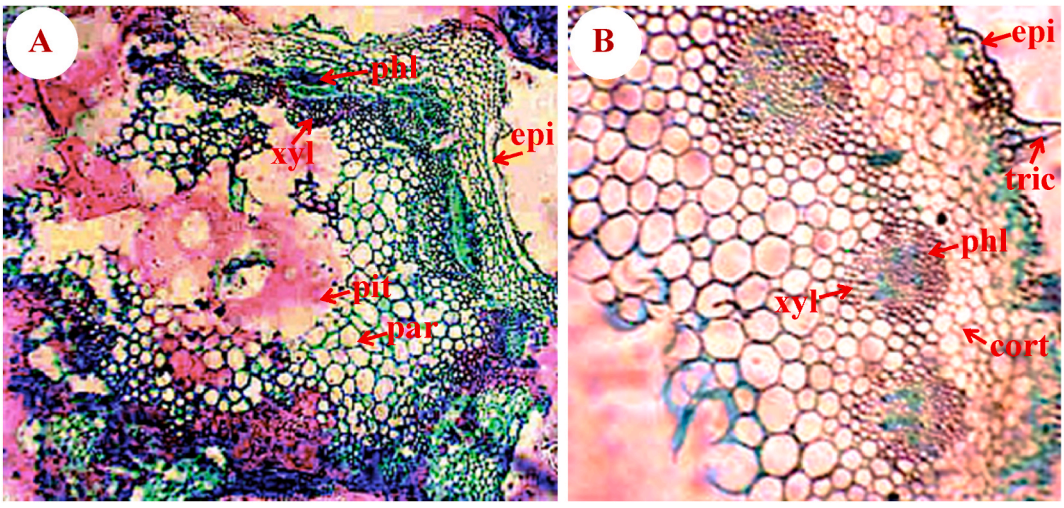

Fig. 2. Microscopic characteristics on transverse section (TS) of the stems; A. *Aster flaccidus* and B. *Aster diplostephioides* (epi-epidermis; phl-phloem; xyl-xylem; pit-pith; par-parenchymatous cells; cort-cortex; tric-trichome).

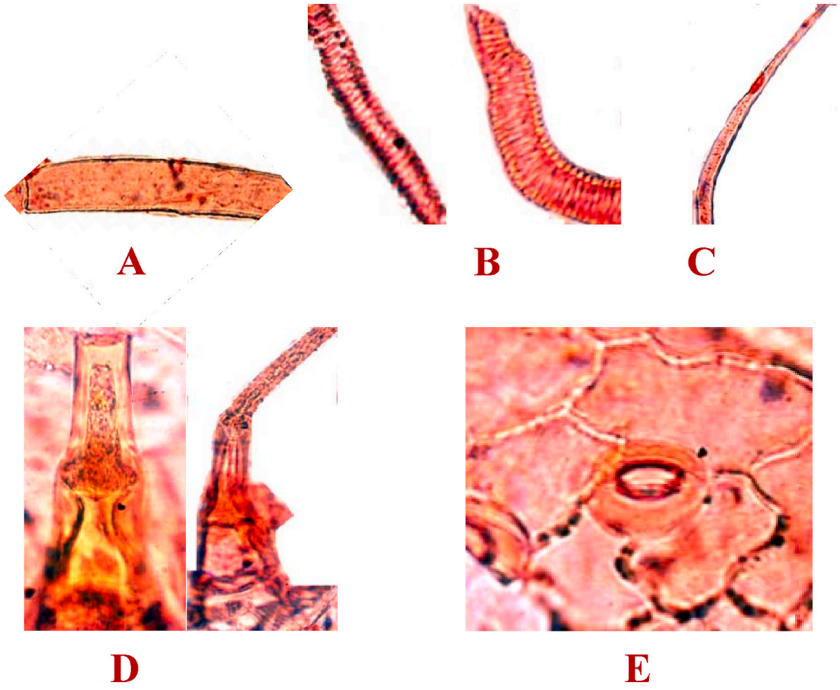

Fig. 3. Isolated elements from crude drug powder of *Aster diplostephioides*: A. tracheids, B. vessels, C. fiber, D. multicellular trichome, E. anomocytic stomata.

Table 3
of ash and extractive values of crude drugs from two *Aster* species.

Parameters	*Aster flaccidus* (≤%)	*Aster diplostephioides* (≤%)
Acid-insoluble ash	8.0	2.0
Alcohol-soluble extractive	7.0	21.0
Foreign matter	2.0	2.0
Loss on drying	10.0	10.0
Total ash	10.0	7.0

The plant parts used in formulating the herbal drugs must be free from physical contaminants, such as soil, sands, stones, harmful foreign matters, including chemical residues, and biological contaminants, including molds, fungi, and insects' excreta [56]. In this study, the foreign matter content in both *Aster* species was 2 %, meeting the acceptable physicochemical limits for crude drugs [53–55]. Additionally, excess water in plant materials will support microbial growth and insects' survival, thus deteriorating the quality of plant materials due to hydrolysis [11]. Therefore, setting a water content limit for all dried plant materials during the quality check process is crucial. The test for loss on drying indicated that both the *Aster* species contained no more than 10 % water or moisture (Table 3).

3.5. HPTLC chromatographic profile of the two Aster species

High-performance thin-layer chromatography (HPTLC) is the most common analytical technique many pharmacopoeias worldwide adopt to assess herbal drugs and preparations. This technique is rapid, simple, robust, and can confirm and be used to establish the identity. The current use is limited to the development of fingerprints for identification, visual observation of chemical markers, and detection of adulterations and falsifications [57]. For this study, we followed the HPTLC method described by Wangchuk and Jamtsho [37–39] to obtain the fingerprints of the two *Aster* species, as shown in Fig. 4 below.

The HPTLC fingerprinting profiles of *A. flaccidus* displayed six bands, while *A. diplostephioides* showed ten bands (Fig. 4). Among them, six bands (Rf 0.23, 0.27, 0.34, 0.42, 0.72, and 0.85) were found to be common in both species under UV light of 366 nm wavelength. However, *A. diplostephioides* exhibited more distinct bands overall, except for the band at Rf 0.72 (dark blue), which was more prominent in *A. flaccidus* (Fig. 4A). On the other hand, under UV light of 254 nm, *A. flaccidus* displayed four bands, while *A. diplostephioides* exhibited five bands, with two bands (at Rf 0.72 and 0.85) in common between the two species. All bands observed under the short wavelength were indistinct, except for the band at Rf 0.34, which was more prominent in *A. diplostephioides* (Fig. 4B). The distinct HPTLC fingerprint presented here will facilitate the differentiation of the two *Aster* species, as it is challenging to

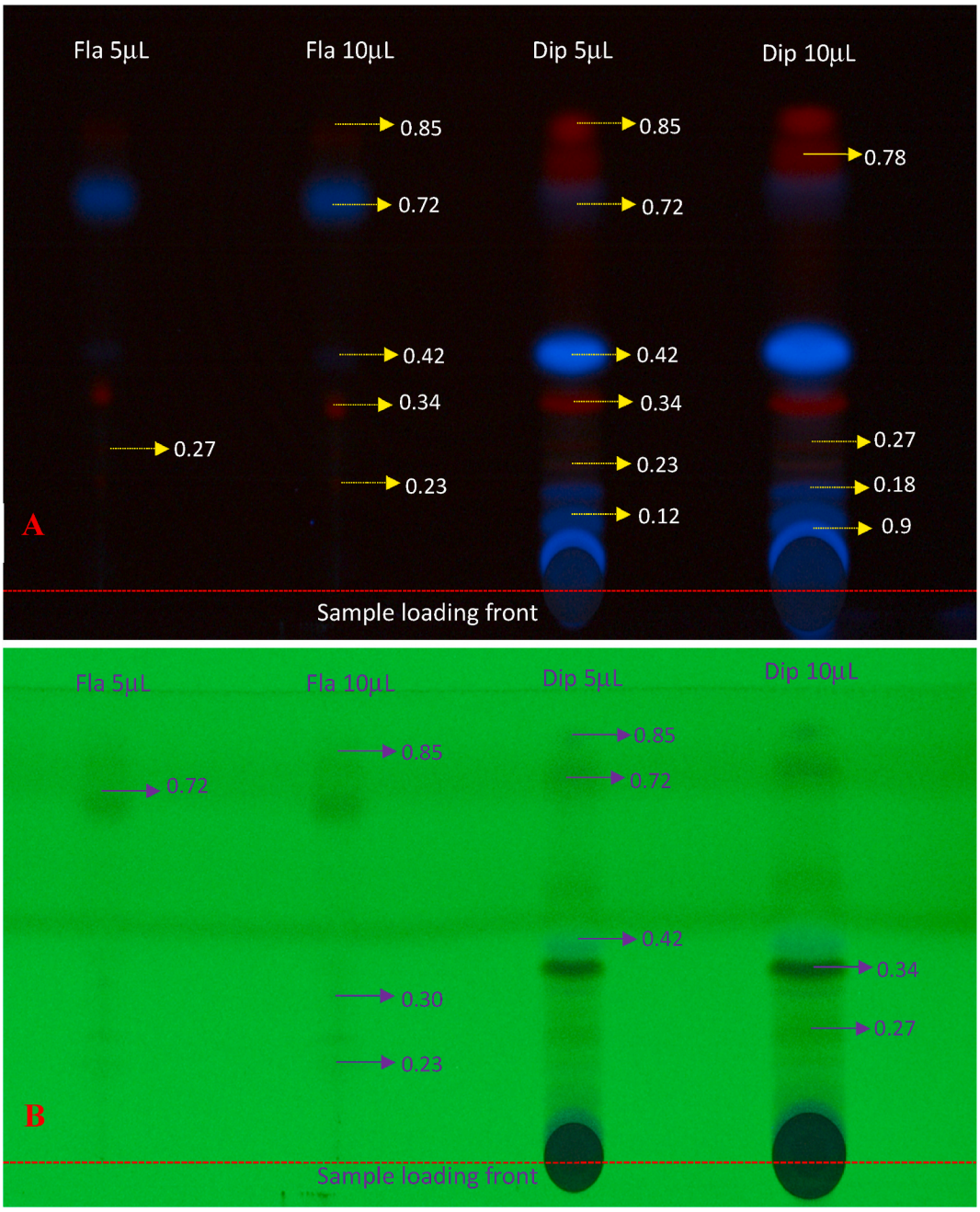

Fig. 4. Chromatogram obtained from the separation of methanolic extracts of two *Aster* species using the solvent system: methanol: chloroform (1: 9) at two different dilutions of 5 µL and 10 µL (Fla 5 µL,10 µL and Dip 5 µL, 10 µL) under UV light wavelengths of 366 nm (A) and 254 nm (B). abbreviations: Fla – *A. flaccidus* and Dip – *A. diplostephioides*.

distinguish two species owing to their similar morphological features and habitat preferences. Moreover, this proposed HPTLC profile can be used as a biochemical marker in the quality check stage to detect potential adulterants in their crude raw materials, thus ensuring the quality and safety of the end products containing these two species as ingredients.

3.6. A literature review on phytochemical constituents and biological properties of two Aster species

3.6.1. Phytochemical constituents

Out of two *Aster* species in this study, only *A. flaccidus* was studied for its phytochemical constituents from its aerial parts and roots. A total of 11 compounds were isolated and identified from aerial parts (9 compounds) and roots (2 compounds) (Table 1). Two new

phenylpropanoids (7′R, 8S)-9′-lariciresinol-(α-methyl)-butanoate; 5,9-dimethoxyl-7-(α-methyl)-butanoxyl-phenyl-2E-propenol-(α-methyl)-butanoate were isolated and identified from the roots [15]. In another study [58], two terpenoid glucosides (2-*O*-β-D-glucopyranoside-vicodiol and 10-*O*-β-D-glucopyranoside-oplopanone) together with seven other known compounds (α-spinasterol, α-spinasterol-β-D-glucopyranoside tetraacetate, alaschanioside A, lariciresinol 9-*O*-β-D-glucopyranoside, alangilignoside D, syringaresinol, and 2,6-dimethoxy-4-(2-propenyl)-phenyl-β-D-glucoside) were isolated from the aerial parts, out of which α-spinasterol and syringaresinol were obtained as major compounds. These major compounds could be used as a chemical marker in HPTLC for assessing the quality of the *A. flaccidus* crude drug. However, phytochemical constituents from the flower used as an ingredient in BSM have not been reported so far.

3.6.2. Biological activities of isolated compounds from Aster flaccidus

We performed a literature search for biological activity reported for crude extracts and compounds obtained from two *Aster* species. There were no bioactivity studies on crude extracts, and out of 11 compounds isolated from *A. flaccidus,* biological activities for only four compounds (Fig. 5) were reported. These four bioactive compounds showed various biological activities, mainly anti-inflammatory and anti-diabetic properties (Table 4). Among four compounds, α-spinasterol was most widely tested in both *in vitro* and *in vivo* studies. It is phytosterol reported in many medicinal plants, and a recent review by Majeed et al. [59] reported α-spinsterol in 31 medicinal plants, including *Aster pseudoglehnii* Y.S. Lim, Hyun & H.Shin. It is also known to be present in most edible plants, including common vegetables such as spinach, cucumber, and pumpkin. α-spinasterol showed anti-inflammatory activity by inhibiting cyclooxygenase enzymes (COX-1 and COX-2) [60] with IC_{50} values of 16.17 μM and 7.8 μM, respectively. The compound also showed a significant reduction ($P < 0.05$) of pro-inflammatory cytokines (tumor necrosis factor, TNF and interleukin 1 beta, IL-1β), prostaglandin E2 (PGE2), and nitric oxide (NO) by a bacterial-derived lipopolysaccharide (LPS)-stimulated cells (BV2 microglial cells and mouse hippocampal HT22 cells) through ERK (extracellular signal-regulated kinase) pathway-dependent expression of HO-1 (heme oxygenase-1) [60]. Syringaresinol also showed similar anti-inflammatory activity in the LPS-stimulated RAW264.7 cells, whereby it inhibited protein and mRNA expressions of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) enzymes and nuclear

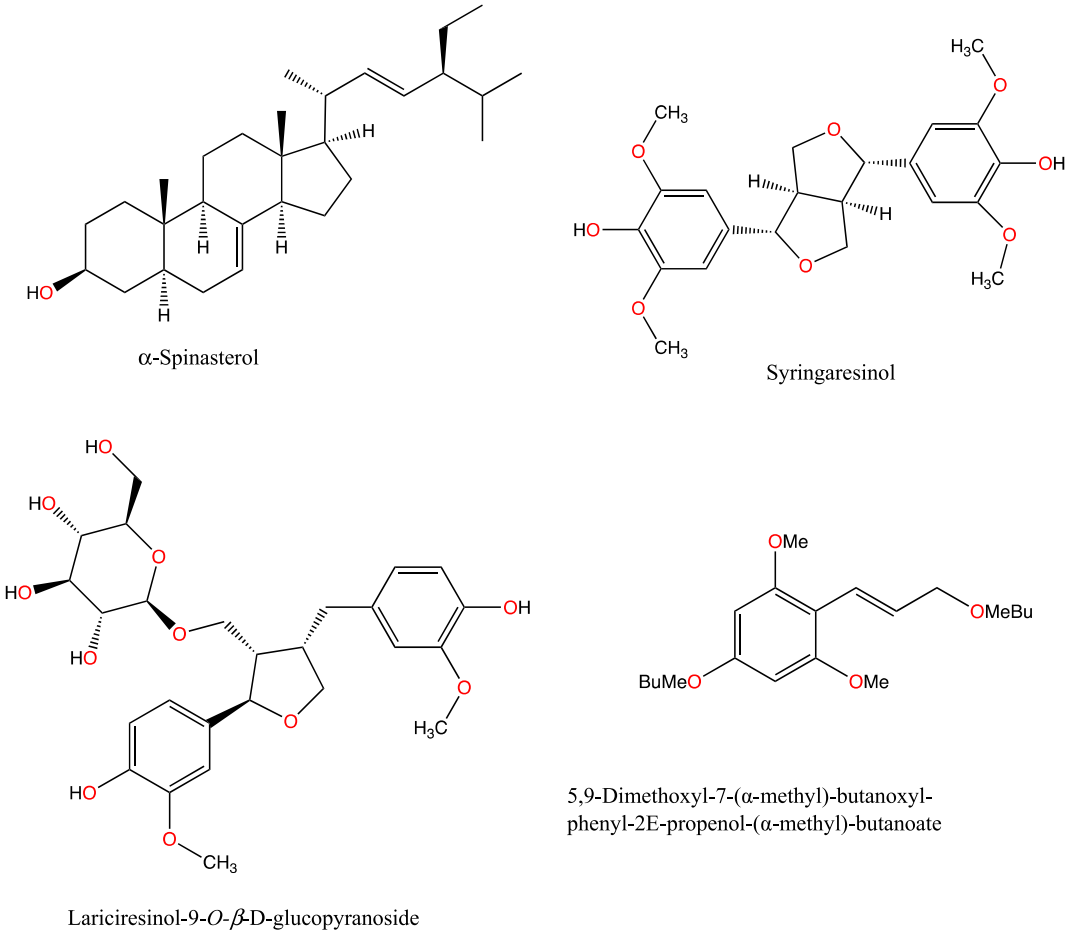

Fig. 5. Chemical structures of bioactive compounds isolated from *Aster flaccidus*.

Table 4
Chemical compounds isolated from different parts of *Aster flaccidus* and their biological activities.

S. No	Compounds	Parts used	Dose	Bioactivity	Mechanism	Models	$IC_{50}/ID_{50}/p$-values
1	2-*O*-β-D-glucopyranoside-vicodiol	Aerial [58]					
2	10-*O*-β-D-glucopyranoside-oplopanone						
3	α-spinasterol		5–100 μg/mL	Anticholinesterase activity [62]	Inhibited the acetylcholinesterase enzyme (obtained from brain homogenate) activity	NA	IC_{50} value, 44.19 ± 2.59 μg/mL
			3–100 μM	Anti-inflammatory [60]	Suppressed the LPS-induced expression of COX-1 and COX-2 activities expressed in prostaglandin levels (in ng/mL)	Male Swiss mice	IC_{50} value, 16.17 μM (COX-1 inhibition) and 7.8 μM (COX-2 inhibition)
			40 μM	Anti-inflammatory [68]	Significantly suppressed the production of NO, PGE2, TNF, and IL-1β by LPS-stimulated cells through ERK pathway-dependent expression of HO-1	BV2 microglial cells and mouse hippocampal HT22 cells	$^*P < 0.05$.
			0.24 μM/kg	Antihyperalgesic activity [66]	Inhibited carrageenan-induced hyperalgesia in mice	Female Swiss mice	42 ± 6 % inhibition
			0.3 μM/kg	Antioedematogenic effect [65]	Inhibited edema produced in capsaicin-induced nociceptive	Male albino Swiss mice	66 ± 10 % inhibition; ID_{50} value, 0.11 μM/kg
			26.93 μg/mL (for DPPH assay); 35.16 μg/mL (for ABTS assay)	Antioxidant [62]	Scavenged DPPH- and ABTS-free radicals	NA	26.93 ± 0.0 % and 35.16 ± 0.26 %
			0.3 μM/kg	Antinociceptive [65]	Inhibited nociception in capsaicin-induced nociceptive mice model	Male albino Swiss mice	58 ± 4 % inhibition
			0.3 mg/kg	Antinociceptive [66]	Inhibited reserpine-induced mechanical allodynia in mice	Male Swiss mice	73 % inhibition
			0.001–10 mg/kg	Antinociceptive [67]	Inhibited acetic acid-induced abdominal constriction	Male Swiss mice	IC_{50}, 99 ± 1 % and ID_{50} value, 0.07 (0.05–0.09 mg/kg)
			50 mg/kg	Anti-ulcer [66]	Reduced the percentage of ulcer	Female Swiss mice	71 %
			5 mg/kg	Antitumour [69]	Reduced testosterone propionate-induced benign prostatic hyperplasia in rats	Wistar-Unilever rats	$P < 0.05$
			15.0 μg/0.2 mL acetone	Antitumour [70]	Reduced the number and incidence of croton oil-induced mouse skin tumours	Swiss Webster albino mice	Reduced incidence of skin tumours by 55.6 % and tumour numbers by 65.0 %.
			10–80 nM/mL	Anti-proliferative [71]	Inhibited cell proliferation	Human colorectal adenocarcinoma CACO-2 cells	60.0 ± 7.10 nM/mL
			12.5–100.0 μg/mL	Anti-proliferative [72]	Inhibited cell proliferation	HeLa and RAW 264.7 cells	IC_{50} values, 77.1 ± 2.1 μg/mL (HeLa cells); 69.2 ± 6 μg/mL (RAW 264.7 cells)
			5–100 μg/mL	Anti-diabetic [62]	Inhibited α-glucosidase enzyme activity	NA	IC_{50} value, 8.65 ± 1.71 μg/mL
			2 mg/kg	Anti-diabetic [73]	Reduced blood sugar level in hyperglycaemic rats	Male Wistar strain rats	22.01 % reduction

(*continued on next page*)

Table 4 (*continued*)

S. No	Compounds	Parts used	Dose	Bioactivity	Mechanism	Models	$IC_{50}/ID_{50}/p$-values
			2.5–10 μM	Anti-diabetic [64]	Increased glucose uptake by mouse skeletal muscle cells Enhanced the secretion of insulin in pancreatic β-cells in response to high glucose	Mouse myoblast C2C12 cells Rat insulinoma INS-1 cells	$P < 0.01$
4	α-spinasterol-β-D-glucopyranoside tetraacetate		NA	NA	NA	NA	NA
5	lariciresinol 9-*O*-β-D-glucopyranoside		NA	Anti-cancer [74]	Cytotoxic against human breast cancer cell lines	Human breast cancer cell lines (Bt549, MCF7, MDA-MB-231 and HCC70)	IC_{50} value, 24.81 μM
6	alaschanioside A			NA	NA	NA	NA
7	alangilignoside D		NA	NA	NA	NA	NA
8	syringaresinol		25–100 μM	Anti-inflammatory [61]	Inhibited protein and mRNA expressions of LPS-stimulated iNOS, COX-2, NF-κB, NO, and PGE2, TNF, IL-1β, and IL-6 production	RAW264.7 cells	$P < 0.01$–0.05
			50 mg/kg	Anti-inflammatory [61]	Reduced carrageenin-induced paw edema volume (mL)	ICR mice	$P < 0.01$
			50 mg/kg		Reduced carrageenin-induced paw edema volume (mL)	ICR mice	$P < 0.01$
			100 μM	Cytotoxicity [75]	Cytotoxic	Hepatoblastoma cells (HepG2) and human colorectal adenocarcinoma (HT29) cells	Non-significant
			NA	Anti-diabetic [63]	Inhibited α-glucosidase enzyme activity	NA	IC_{50} value, 19.5 ± 0.2 μg/mL
			20 μM	Antiphotoaging [76]	Inhibited UVA-induced upregulation of MMP-1 by suppressing MAPK/AP-1 signaling and enhanced collagen production	UVA-irradiated human HaCaT keratinocytes and dermal fibroblasts (HDFs)	Reduced the level of MMP-1 (induced by UVA) from 8.16 ng/mL to 2.56 ng/mL and from 2.28 ng/mL to 1.37 ng/mL in HaCaT keratinocytes and HDFs, respectively.
9	2,6-dimethoxy-4-(2-propenyl)-phenyl-β-D-glucoside		NA	NA	NA	NA	NA
10	(7′R, 8S)-9′-lariciresinol-(α-methyl)-butanoate	Roots [15]	NA	NA	NA	NA	NA
11	5,9-dimethoxyl-7-(α-methyl)-butanoxyl-phenyl-2E-propenol-(α-methyl)-butanoate		NA	Anti-tumour [15]	Exhibited anti-cancer activity against human liver carcinoma cells	Human liver carcinoma, BEL 7402 cells	IC_{50} values, 106.67 ± 8.47 μM at 24 h, 50.51 ± 6.11 μM at 72 h

In the "dose" column, "mg/kg, μM/kg" represents the oral/parenteral dose of the compound in mice/rats; concentration units (μg/mL, nM/mL) represent the concentration of the compound in the cell treatment. ABTS - 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid; COX – cyclo-oxygenase; DPPH – 2,2-diphenyl-1-picrylhydrazyl; ERK – extracellular signal-regulated kinase; HaCaT – cultured human keratinocyte (cells); HeLa – Lack's cervical cancer cells; HO-1 – heme oxygenase-1; IC_{50} – the half-maximal inhibitory concentration; ID_{50} – infectious dose 50; ICR – institute of cancer research; IL – interleukins; iNOS – inducible nitric oxide synthase; LPS – lipopolysaccharide; MMP-1 – matrix metalloproteinases; MAPK/AP-1 – mitogen-activated protein kinases (MAPK)/activator protein-1 (AP-1); mRNA – messenger ribonucleic acid; NA – not available/not tested; NF-κB – nuclear factor kappa B; NO – nitric oxide; PGE2 – prostaglandins E2; RAW 264.7 – macrophage-like, Abelson leukemia virus-transformed cell line derived from Balb/c mice; TNF – tumour necrosis factor.

factor kappa B (NF-κB) pathway [61]. Nitric oxide, PGE2, TNF, IL-1β, and IL-6 productions by LPS-stimulated RAW264.7 cells were also significantly ($p < 0.01-0.05$) suppressed by syringaresinol [61].

Both α-spinasterol and syringaresinol also exhibited anti-diabetic properties mainly by inhibiting α-glucosidase enzyme activity [62,63]. Alpha-spinosterol also enhanced insulin secretion by pancreatic β-cells of the rat insulinoma INS-1 cells in response to high glucose concentration [64]. These compounds also exhibited other activities, including anti-tumour, anti-nociceptive, neuroprotective, and anti-aging properties (Table 4). Lariciresinol 9-*O*-β-D-glucopyranoside exhibited anticancer activities against human breast cancer.

In BTM, traditional formulations, *a-gar-35* and *rin-chen-byur-d.mar-25*, contain two *Aster* species as an ingredient are indicated for pain and neurological disorders (Table 1). For instance, α-spinasterol showed neuroprotection by inhibiting an acetylcholinesterase enzyme (obtained from brain homogenate) activity [62] and anti-nociceptive activity in various mice models (Table 4) [65–67]. Thus, this compound could be responsible for beneficial effects against pain and neurological disorders in BSM medication. It would be interesting to analyze *a-gar-35* and *rin-chen-byur-d.mar-25* formulations for the presence of such bioactive compounds (Fig. 5) using modern techniques such as high-performance liquid chromatography-mass spectrometry (UPLC-MS/MS).

3.7. Species-specific features and the potential checklist for authenticating two Aster species

The BTM system relies on morphological attributes, habitat preferences, and biogeographical and ecological distribution patterns as routine traditional methods to identify and collect medicinal plants. This study examined two related *Aster* species, *A. flaccidus* and *A. diplostephioides*, and identified species-specific characteristics/traits/features (Table 5) that can be used as a checklist to authenticate two species. Morphologically, *A. flaccidus* can grow up to 3–30 (−40) cm tall and has long and slender rhizomes, while *A. diplostephioides* typically reach heights of 13–37 cm and have robust and branched rhizomes. Anatomically, the T.S. of the *A. flaccidus* stem typically appears hexagonal or octagonal with distinct ridges on the surface. Their cortical zone is composed of parenchymatous cells, with 4–5 layers of cells, while at the ridges, 8–10 layers are present. The T.S. of the *A. diplostephioides* stem was circular, with a single layer of parenchymatous cells without intercellular spaces, and the cortex consists of 3–5 layers of parenchyma cells. The two *Aster* species also exhibited variations in the physicochemical parameters of their crude drugs. For example, the total ash

Table 5
Comparative distinguishing characteristics of two *Aster* species.

Parameters	Distinctive features	*Aster flaccidus*	*Aster diplostephioides*
Ecological	*Sowa Rigpa* name (local name)	lug-mig	a-byag-g.zer-'joms
	Habitat	Sandy slopes, grasslands, pastures, meadows, thickets, screes, and fallow fields	Scrubland, marshy areas, riverine, and floodplains
	Distribution within Bhutan	Thimphu (Lingzhi), Paro (upper Paro Chu-Gafoo La), Bumthang (Marlung), and Gasa	Thimphu (Lingzhi), Bumthang, Gasa, Haa, and Punakha
	Altitude	3750−5025 masl	3500−4200 masl
Morphological	Plant height	3−30 (−40) cm	13−57 cm
	Rhizome	Long and slender	Robust and branched
	Stem types	Glandular (5−15 cm)	Scapiform (15−40 cm)
	Leaf shape and size	Basal leaves spathulate or oblanceolate (1.7−6.5 × 0.8−1.5 cm)	Basal leaves lanceolate or oblanceolate (6−13 × 1.3−2 cm)
		Cauline leaves narrowly lanceolate	Cauline leaves oblong-linear
	Involucre type and diameter	2−3 seriate, 12−17 mm	±2-seriate, 17−22 mm
	Phyllaries	Lanceolate (8−10 × 1.5−2 mm)	Oblong-lanceolate (10−12 × 3−4.5 mm)
	Ray florets	31−67 flowers, blue or mauve, rarely pink or pinkish white	45−93 flowers, mauve to purple or lilac-blue
	Disc florets	Yellow corollas	Blackish-purple fading to orange corollas
	Pappus	4-seriate, whitish (sometimes purplish)	3-seriate, white
Anatomical	Transverse section of stem	Appears hexagonal or octagonal with distinct ridges on the surface	Appears circular, with a single layer of parenchymatous cells without intercellular spaces
		Cortex zone comprises 4−5 layers of cells, while 8−10 layers are present at the ridges	The cortex is composed of 3–5 layers of parenchyma cells
Physicochemical	Total ash	≤10 %	≤7 %
	Alcohol-insoluble extractive	≤7 %	≤21 %
Traditional medicine	Number of formulations	3	1
	Parts used	Stem and flowers	Leaves
	Diseases treated	Chronic fever, cardiovascular diseases, cold, arthritis, insomnia, dehydration, stomach disorder, and loss of appetite	Neurological disorder, giddiness, neck stiffness, and chronic headache
HPTLC profile	Under 366 nm UV light	6 bands, dark blue with Rf 0.72, is the most distinct	10 bands, fluorescent blue at Rf 0.42 is the most distinct
	Under 254 nm UV light	4 bands	5 bands with Rf 0.34 band as the most distinct

Abbreviations: HPTLC – high-performance thin layer chromatography; masl – meters above sea level; Rf – retention factor; UV – ultraviolet.

value of *A. flaccidus* (10 %) crude drug was higher than that of *A. diplostephioides* (7 %). However, alcohol-insoluble extractive value (21 %) was higher in *A. diplostephioides* crude drug than in *A. flaccidus* (7 %).

The HPTLC profiles of the methanolic crude extracts of two *Aster* species were also distinct from typical chromatograms, as described in Fig. 4 and Table 5. Since chemical isolation and biological testing are lengthy and expensive, MSPCL currently uses this HPTLC profiling technique to monitor the quality of the raw materials of medicinal plants by comparing them with the standard samples. Using major chemicals isolated and identified from two *Aster* species (e.g., α-spinasterol and syringaresinol from *A. flaccidus*) as a chemical marker to develop HPTLC chromatograms could serve as a more reliable and authentic fingerprint for the quality assessment.

3.8. Advancement in the field of medicinal plants research and future application for monitoring the quality of herbal medicine in Bhutan

The quality of BTM formulations may vary significantly due to differences in medicinal plants' geographical locations, genetic backgrounds, harvesting timing, and many other factors, including exogenous impurities. Unlike chemical drugs, maintaining quality consistency for poly-ingredient herbal drugs is challenging because their complex chemical compositions and active ingredients cannot be detected easily [77,78]. Poor and inconsistencies in quality assessment may not guarantee the drug's effectiveness, increasing their safety risk. With the emergence of many advanced analytical technologies, more attention has been given to ensuring the quality consistency of herbal drugs in many countries where traditional medicines are still used as complementary therapeutics [79]. Generally, three common approaches are employed, namely i) chemical evaluation, ii) biological evaluation, and iii) an integrated approach of these two to maintain the chemical or biological homogeneity of herbal drugs [77].

For the chemical evaluation, chromatographic and spectral evaluation methods are applied using liquid chromatography (LC), gas chromatography (GC), and mass spectrometry (MS). Among the chromatographic techniques, high-performance liquid chromatography (HPLC) and reverse-phase HLPC (RP-HPLC) are considered highly convenient and reproducible techniques to estimate bioactive chemical markers in the poly-ingredient herbal products both qualitatively and quantitatively [80,81]. Another chromatography technique that is more rapid and eco-friendly is supercritical fluid chromatography (SFC). This technique can also detect and quantify various chemical markers, including fat-soluble vitamins. Unlike the HPLC method, SFC uses compressed CO2 with small quantities of organic solvents [80]. Recently, ultra-high performance liquid chromatography (UPLC) has gained popularity in analyzing herbal drugs [82]. Liquid chromatography combined with mass spectrometry (HPLC/MS/MS) can determine multiple components of herbal drugs. For instance, Tang et al. [83] determined 14 toxic and effective components in Fuzi formula granules of traditional Chinese medicine (TCM) and their variations among 29 batches from two manufacturers. Another similar approach is using HPLC in combination with quantitative analysis of multiple components by a single marker (QAMS) [78]. This method (HPLC fingerprint combined with QAMS) is reliable in accuracy and consistency even without standard/reference compounds to determine the composition of complex herbal formulations [84].

The spectral evaluation technique is also a rapid method to analyze the composition of herbal formulations. One such example is near-infrared (NIR) spectroscopy, a computer-assisted technology for analysis and testing without requiring sample pretreatments [85], and it works with mathematical models of the samples using stoichiometry software [86,87]. Near-infrared spectroscopy combined with multivariate statistical analyses can monitor the quality of the herbal drug during extraction and production online in real time for multiple batches, significantly improving the quality of herbal medicines [88]. Such computer-assisted techniques can be established as they require only training human resources. They would be much cheaper than installing expensive analytical instruments such as nuclear magnetic resonance (NMR) and LC/GC-MS, where maintenance costs are unimaginable for developing countries like Bhutan. Nuclear magnetic resonance-based, GC-MS-based, and gas chromatography-time-of-flight-mass spectrometry (GC-MS-TOF)-based metabolomics are also widely used quality control tools for herbal products [89]. Raman spectroscopy can detect the mineral composition in the herbal drug [90], and a modern portable and handheld version of this instrument is widely used for detecting the low levels of metal compositions in the drug [91]. Such a technique is a necessity for BSM quality assurance since BSM currently uses 28 minerals in various herbo-mineral formulations [92], as contaminations with toxic metals like arsenic and mercury will be deleterious to patients' health. The research unit under the MSCPL has started using inductively coupled plasma mass spectrometry (ICP-MS) to detect trace metal elements in raw materials; however, they cannot utilize the facility routinely due to a lack of skilled human resources, which requires immediate attention. The ICP-MS can detect traces of chemical elements such as manganese, strontium, aluminium, magnesium, iron, zinc, and lead [93].

Establishing the quality control for maintaining biological consistency is challenging for resource-constraint developing countries. However, MSPCL must include WHO-recommended testing for determining microbial toxins, microbiological contaminants, such as *Salmonella* and *Shigella* species, and pesticide residues. For instance, the maximum limit of pesticide residues as per the WHO guidelines [94] is not more than 1 %. A recently developed bioassay method called 'microcalorimetry' can be used to develop bioactivity fingerprints by analyzing the interaction between the drug and the organism using mathematical modeling [95].

Advancements in deoxyribonucleic acid (DNA) sequencing technology and bioinformatics tools have also opened a new platform for the quality control of herbal drugs through developing DNA barcodes, and it is considered a rapid and reliable method for identifying medicinal herbs, starting in 2003 [96]. The invention of next-generation sequencing (NGS) technology and the emergence of third-generation sequencing have further empowered the development of DNA barcode-derived techniques for identifying herbal medicinal species. These techniques include super-barcoding, *meta*-barcoding, and mini-barcoding, each serving specific purposes. For instance, mini-barcoding identifies species from highly degraded DNA [97,98], while *meta*-barcoding helps analyze species richness in a sample containing a mixture of species [99,100]. On the other hand, super-barcoding, based on the plant chloroplast genome, helps determine species-relatedness [101,102]. However, it is important to note that these advanced DNA-based techniques may be costly. In

such a scenario, collaborating with countries with access to such technologies is the best option for MSCPL.

4. Conclusions and future perspectives

Traditionally, BTM uses rely on habitat preferences and taxonomical features to identify medicinal plants. However, this technique cannot distinguish closely related medicinal plant species and various adulterants/physical contaminants, particularly once plants are dried and made into powder form. Therefore, to strengthen the existing practice, the current study employed an integrated approach of traditional ethnopharamacognostic and modern pharmacognostic evaluations to determine key morphological, anatomical, and physicochemical parameters, including HPTLC fingerprints for two *Aster* species for the first time. Both species (*Aster flaccidus* and *A. diplostephioides*) possessed distinct morphological and anatomical features and physicochemical parameters, including the HPTLC chromatogram. Physicochemical parameters and HPTLC fingerprints obtained in this study would help enhance the efficiency and safety of the existing routine quality assessment of these *Aster* species used as ingredients in BSM formularies.

Phytochemically, through literature review, out of two species, only *A. flaccidus* was found to be studied, from which 11 compounds were isolated from its aerial parts and roots. However, no phytochemical studies have been reported on its flowers, which are used as an ingredient in BSM formulations. Thus, future studies should focus on analyzing the phytochemical constituents of flowers of *A. flaccidus* and aerial parts/stems of *A. diplostephioide* to develop chemical markers for the quality control. Although the parameters established in this study would be adequate to monitor the quality of two *Aster* species, developing more authentic and reliable parameters through collaborations using the latest molecular techniques, such as DNA barcoding and metabolomics profiling, would be better. Menjong Sorig Pharmaceutical Corporation Limited has already started investing in modern technologies for quality control, such as inductively coupled plasma mass spectrometry (ICP-MS), which can detect trace heavy metals in raw materials. They must also incorporate other modern testing systems, including screening tests for pests, pesticides, and microbial contaminations in crude drugs, which is currently lacking in their quality assessment. Conducting toxicity tests for raw ingredients and finished products and conducting clinical trials for the prescribed medications using BSM formulations would further support its efficacy and safety.

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Data availability

All data are included in this manuscript.

CRediT authorship contribution statement

Ngawang Gempo: Writing – review & editing, Writing – original draft, Methodology. **Karma Yeshi:** Writing – review & editing, Writing – original draft, Validation, Formal analysis, Data curation. **Tenzin Jamtsho:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lungten Jamtsho:** Visualization, Validation, Investigation, Data curation. **Samten:** Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Phurpa Wangchuk:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] P. Wangchuk, T. Tobgay, Contributions of medicinal plants to the gross national happiness and biodiscovery in Bhutan, J. Ethnobiol. Ethnomed. 11 (1) (2015) 48.
[2] M.H. Al Rashid, et al., Preclinical and clinical trials of Indian medicinal plants in disease control, in: S. Sen, R. Chakraborty (Eds.), Herbal Medicine in India: Indigenous Knowledge, Practice, Innovation and its Value, Springer Singapore, Singapore, 2020, pp. 119–142.
[3] K. Yeshi, et al., An integrated medicine of Bhutan: Sowa Rigpa concepts, botanical identification, and the recorded phytochemical and pharmacological properties of the eastern Himalayan medicinal plants, European Journal of Integrative Medicine 29 (2019) 1–15.
[4] P. Wangchuk, T. Gyeltshen, T. Tobgay, Bhutanese traditional medical education, in: M.J. Schuelka, T.W. Maxwell (Eds.), Education in Bhutan: Culture, Schooling, and Gross National Happiness, Springer Singapore, Singapore, 2016, pp. 91–108.
[5] 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.

[6] 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 Compl. Alternative Med. 16 (2016) 283.
[7] G. Prinsloo, N. Nogemane, The effects of season and water availability on chemical composition, secondary metabolites and biological activity in plants, Phytochemistry Rev. 17 (4) (2018) 889–902.
[8] Y. Li, C. Zidorn, Seasonal variations of natural products in European herbs, Phytochemistry Rev. 21 (5) (2022) 1549–1575.
[9] A.F. Gomes, et al., Seasonal variation in the chemical composition of two chemotypes of Lippia alba, Food Chem. 273 (2019) 186–193.
[10] P. Wangchuk, Samten, T. Jamtsho, Phytopharmaceutical properties and quality assessment of two Himalayan medicinal plants, Meconopsis horridula and Meconopsis simplicifolia, J. Herb. Med. 38 (2023) 100628.
[11] WHO, Quality Control Methods for Medicinal Plant Materials, WHO Library, Geneva, 1998.
[12] WHO, Quality Assurance of Pharmaceuticals: a Compendium of Guidelines and Related Materials. Good Manufacturing Practices and Inspection, 2, World Health Organization, 2007.
[13] Joint, F. and W.E.C.o.F. Additives, WHO Technical Report Series, WHO, Geneva, 1989.
[14] S. Hazarika, S. Hemalatha, Quality control assessment, toxicity profiling, and experimental validation of network pharmacology-predicted anti-inflammatory potential of Natsiatum herpeticum Buch.-Ham. Ex Arn, J. Ethnopharmacol. 318 (2024) 116902.
[15] Z.L. Liu, et al., The phenylpropanoids of Aster flaccidus, Fitoterapia 81 (2) (2010) 140–144.
[16] POWO., Plants of the World Online, Facilitated by the Royal Botanic Gardens, Kew, 2023. *Published on the Internet;*, http://www.plantsoftheworldonline.org/.
[17] Y. Shao, et al., Triterpenoid saponins from Aster lingulatus, Phytochemistry 44 (2) (1997) 337–340.
[18] Y. Shao, et al., Aster lingulatosides C and D, cytotoxic triterpenoid saponins from Aster lingulatus, J. Nat. Prod. 60 (7) (1997) 743–746.
[19] C. Wang, D. Yu, Triterpenoid saponins from Aster auriculatus, Planta Med. 64 (1998) 50–53.
[20] A.J.C. Grierson, D.G. Long, Flora of Bhutan: Including a Record of Plants from Sikkim, 1, Royal Botanic Garden, Edindurgh, 1984. Part 2.
[21] D. Phuntshok, Shel-gong-shel-phreng, Tibetan Medical & Astro Institute, 1994 W3JT13436.
[22] P. Wangchuk, Samten, Monographs on Medicinal Plants of Bhutan, 2, Pharmaceutical and Research Unit, Ministry of Health, Thimphu, Bhutan, 2009.
[23] A.J. Grierson, D.G. Long, Flora of Bhutan: Including a Record of Plants from Sikkim, Royal Botanic Garden Edinburgh, Edinburgh, 1983.
[24] S. Zhu, et al., Asteraceae (Compositae), family introduction, glossary, systematic list, and key to tribes, in: Z.Y. Wu, P.H. Raven, D.Y. Hong (Eds.), Flora of China, Science Press (Beijing) & Missouri Botanical Garden Press, St. Louis): Beijing, China, 2011, pp. 1–8.
[25] P. Wangchuk, et al., Medicinal plants of Dagala region in Bhutan: their diversity, distribution, uses and economic potential, J. Ethnobiol. Ethnomed. 12 (1) (2016) 28.
[26] P. Wangchuk, Samten, GC-FID coupled GC-MS analysis of the essential oil and the recorded biological activities ofMeconopsis simplicifolia, Journal of Biologically Active Products from Nature 5 (6) (2016) 365–372.
[27] P. Wangchuk, K. Yeshi, K. Jamphel, Pharmacological, ethnopharmacological, and botanical evaluation of subtropical medicinal plants of Lower Kheng region in Bhutan, Integr Med Res 6 (4) (2017) 372–387.
[28] D.A. Johansen, Plant Microtechnique Ied, McGraw-Hill, New York, 1940.
[29] P. Wangchuk, et al., Three medicinal Corydalis species of the Himalayas: their ethnobotany, pharmacognosy, phytochemistry and pharmacology, J. Herb. Med. 23 (2020) 100384.
[30] Samten, Monographs on Medicinal Plants of Bhutan, 1, Pharmaceutical and Research Unit, Ministry of Health, Thimphu, Bhutan, 2009.
[31] Anonymous, Thai Herbal Pharmacopoeia, 2, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand, 2000.
[32] P. Wangchuk, et al., Monographs on Medicinal Plants of Bhutan, Ministry of Health, Bhutan, 2008.
[33] J. Who, F.E. Consultation, Diet, nutrition and the prevention of chronic diseases, World Health Organ Tech Rep Ser 916 (i–viii) (2003) 1–149.
[34] Anonymous, Thai Herbal Pharmacopoeia, 1, Ministry of Public Health, Bangkok, Thailand, 1990.
[35] WHO, WHO Monographs on Selected Medicinal Plants, 2, World Health Organisation, Geneva, 2002.
[36] WHO, Quality Control Methods for Herbal Materials (*Updated Edition of Quality Control Methods for Medicinal Plant Materials*, World Health Organization, 2011.
[37] T. Jamtsho, K. Yeshi, P. Wangchuk, Comparative analysis of two Himalayan Aconitum species for their phytopharmaceutical properties, J. Herb. Med. 32 (2022) 100497.
[38] P. Wangchuk, T. Jamtsho, Phytopharmaceutical properties and quality assessment of two Himalayan medicinal plants, Meconopsis horridula and Meconopsis simplicifolia, J. Herb. Med. 38 (2023) 100628.
[39] P. Wangchuk, T. Jamtsho, Inula racemosa hook. F. Pushkarmool: its ethnobotanical uses, phytochemicals, and pharmacological activities, in: Immunity Boosting Medicinal Plants of the Western Himalayas, Springer, 2023, pp. 253–289.
[40] S. Tenzin, Traditional Medicine Formulary of Bhutan, Pharmaceutical and Research Unit, Institute of Traditional Medicine Services, Department of Medical Services, Ministry of Health, Thimphu, Bhutan, 2007.
[41] P. Wangchuk, et al., Medicinal plants of Dagala region in Bhutan: their diversity, distribution, uses and economic potential, J. Ethnobiol. Ethnomed. 12 (2016) 1–19.
[42] S. Tenzin, S. Tashi, Monograph on Traditional Medicine of Bhutan, Menjong Sorig Pharmaceuticals, Department of Traditional Medicine Services, Ministry of Health, Thimphu, 2015.
[43] S. Bhattarai, R.P. Chaudhary, R.S. Taylor, Ethnomedicinal plants used by the people of Manang district, central Nepal, J. Ethnobiol. Ethnomed. 2 (2006) 41.
[44] Z.-l. Liu, et al., The phenylpropanoids of Aster flaccidus, Fitoterapia 81 (2) (2010) 140–144.
[45] C. Barooah, I. Ahmed, Plant Diversity of Assam (A Checklist of Angiosperms and Gymnosperms), ASTEC. Bigyan Bhawan, Guwahati, Assam, 2014.
[46] Y. Roskov, et al., *Species 2000 & ITIS catalogue of life.* 2019 annual checklist, SPECIES (2000) 2405–2884. Digital resource at, www.catalogueoflife.org/annual-checklist/2019.
[47] WFO, Aster Diplostephioides (DC, Benth. ex C.B.Clarke., 2023. Available from: Published on the Internet, http://www.worldfloraonline.org/taxon/wfo-0000026882.
[48] S.E. Ruzin, Plant Microtechnique and Microscopy, 198, Oxford University Press, New York, 1999.
[49] A. Hadidchi, F. Attar, F. Ullah, Using microscopic techniques for taxonomic implications of seed and fruits of Delphinium L.(sensu lato)(Ranunculaceae), Microsc. Res. Tech. 83 (2) (2020) 99–117.
[50] K. Yeshi, et al., An integrated medicine of Bhutan: Sowa Rigpa concepts, botanical identification, and the recorded phytochemical and pharmacological properties of the eastern Himalayan medicinal plants, European Journal of Integrative Medicine 29 (2019) 100927.
[51] S. Mukhi, et al., Pharmacognostic, physicochemical and chromatographic characterization of Samasharkara Churna, J. Ayurveda Integr. Med. 7 (2) (2016) 88–99.
[52] H.S. Chandel, A.K. Pathak, M. Tailang, Standardization of some herbal antidiabetic drugs in polyherbal formulation, Pharmacogn. Res. 3 (1) (2011) 49–56.
[53] Anonymous, Thai *herbal pharmacopoeia (1)*, Depart. Med. Sci. 1 (1995) 51–56. Thailand.
[54] WHO, Quality Control Methods for Herbal Materials, World Health Organization, Geneva, 2011.
[55] Anonymous, Thai Herbal Pharmacopoeia 2017, The Agricultural Co-operative Federation of Thailand Bangkok, 2017.
[56] M. Nafiu, et al., Preparation, Standardization, and Quality Control of Medicinal Plants in Africa, Medicinal spices and vegetables from Africa, 2017, pp. 171–204.
[57] S. Lamichhane, et al., Herbal drug standardization: a systemic review, J. Drug Deliv. Therapeut. 13 (4) (2023) 149–153.
[58] L.S. Gan, et al., Two new terpenoid glucosides from Aster flaccidus, J. Asian Nat. Prod. Res. 8 (7) (2006) 589–594.
[59] M. Majeed, et al., Pharmacology of alpha-spinasterol, a phytosterol with nutraceutical values: a review, Phytother Res. 36 (10) (2022) 3681–3690.
[60] I. Brusco, et al., α-Spinasterol: a COX inhibitor and a transient receptor potential vanilloid 1 antagonist presents an antinociceptive effect in clinically relevant models of pain in mice, Br. J. Pharmacol. 174 (23) (2017) 4247–4262.

[61] V.K. Bajpai, et al., Attenuation of inflammatory responses by (+)-syringaresinol via MAP-Kinase-mediated suppression of NF-kappaB signaling in vitro and in vivo, Sci. Rep. 8 (1) (2018) 9216.
[62] B.A. Lawal, et al., Novel cholinesterase inhibitory effect of α-spinasterol isolated from the leaves of Acacia auriculiformis A. Cunn Ex. Benth (Fabaceae), Trop. J. Pharmaceut. Res. 19 (7) (2020) 1473–1479.
[63] T.V.L. Nguyen, et al., α-glucosidase inhibitory activity of extracts and compounds from the leaves of Ruellia tuberosa L, Nat. Prod. J. 12 (5) (2022) e180221191577.
[64] D. Lee, et al., Dual beneficial effects of alpha-spinasterol isolated from aster pseudoglehnii on glucose uptake in skeletal muscle cells and glucose-stimulated insulin secretion in pancreatic beta-cells, Plants 11 (5) (2022).
[65] G. Trevisan, et al., Identification of the plant steroid alpha-spinasterol as a novel transient receptor potential vanilloid 1 antagonist with antinociceptive properties, J. Pharmacol. Exp. Therapeut. 343 (2) (2012) 258–269.
[66] L.C. Klein-Júnior, et al., Antihyperalgesic activity of the methanol extract and some constituents obtained from Polygala cyparissias (Polygalaceae), Basic Clin. Pharmacol. Toxicol. 111 (3) (2012) 145–153.
[67] F.C. Meotti, et al., Antinociceptive properties of coumarins, steroid and dihydrostyryl-2-pyrones from Polygala sabulosa (Polygalaceae) in mice, J. Pharm. Pharmacol. 58 (1) (2006) 107–112.
[68] G.S. Jeong, et al., Cytoprotective and anti-inflammatory effects of spinasterol via the induction of heme oxygenase-1 in murine hippocampal and microglial cell lines, Int. Immunopharm. 10 (12) (2010) 1587–1594.
[69] M.Y. Lee, et al., α-spinasterol from Melandrium firmum attenuates benign prostatic hyperplasia in a rat model, Mol. Med. Rep. 9 (6) (2014) 2362–2366.
[70] I.M. Villaseñor, A.P. Domingo, Anticarcinogenicity potential of spinasterol isolated from squash flowers, Teratog. Carcinog. Mutagen. 20 (3) (2000) 99–105.
[71] Y.S. Ravikumar, et al., Antiproliferative, apoptotic and antimutagenic activity of isolated compounds from Polyalthia cerasoides seeds, Phytomedicine 17 (7) (2010) 513–518.
[72] S.E. Meneses-Sagrero, et al., Antiproliferative activity of spinasterol isolated of Stegnosperma halimifolium (Benth, 1844), Saudi Pharmaceut. J. 25 (8) (2017) 1137–1143.
[73] N.Y. Htwe, M.M. Thu, W. Naing, Isolation of bioactive compounds from bark of Mimusops elengi Roxb. (Kha-yay) used in the treatment of hyperglycemia, Journal of Myanmar Academy of Arts and Science 18 (2020) 191–199.
[74] J. Baek, et al., Lignan glycosides and flavonoid glycosides from the aerial portion of lespedeza cuneata and their biological evaluations, Molecules 23 (8) (2018).
[75] V. Kirsch, T. Bakuradze, E. Richling, Toxicological testing of syringaresinol and enterolignans, Curr Res Toxicol 1 (2020) 104–110.
[76] J.H. Oh, et al., Syringaresinol inhibits UVA-induced MMP-1 expression by suppression of MAPK/AP-1 signaling in HaCaT keratinocytes and human dermal fibroblasts, Int. J. Mol. Sci. 21 (11) (2020).
[77] X.-C. Wei, et al., Recent advances of novel technologies for quality consistency assessment of natural herbal medicines and preparations, Chin. Med. 15 (1) (2020) 56.
[78] D.K. Zhang, et al., Investigation of the chemical markers for experiential quality evaluation of crude aconite by UHPLC-Q-TOF-MS, J. Separ. Sci. 39 (22) (2016) 4281–4289.
[79] X.-R. Xu, et al., Overview and prospects of traditional Chinese medicine blending technology oriented by quality consistency, Zhongguo Zhong yao za zhi= Zhongguo zhongyao zazhi= China journal of Chinese materia medica 44 (22) (2019) 4786–4792.
[80] A. Balekundri, V. Mannur, Quality control of the traditional herbs and herbal products: a review, Future Journal of Pharmaceutical Sciences 6 (1) (2020) 67.
[81] R. Govindarajan, V. Tejas, P. Pushpangadan, High-performance liquid chromatography (HPLC) as a tool for standardization of complex herbal drugs, J. AOAC Int. 102 (4) (2019) 986–992.
[82] S. Zeng, et al., Monitoring batch-to-batch reproducibility using direct analysis in real time mass spectrometry and multivariate analysis: a case study on precipitation, J. Pharm. Biomed. Anal. 76 (2013) 87–95.
[83] J.-F. Tang, et al., Quality consistency evaluation of Fuzi formula granules using determination of multi-component contents by HPLC-MS/MS method, Zhongguo Zhong yao za zhi= Zhongguo zhongyao zazhi= China journal of Chinese materia medica 43 (9) (2018) 1871–1879.
[84] F. Li, et al., Quantitative analysis of multi-components by single marker and fingerprint analysis of Achyranthes bidentata blume, J. Chromatogr. Sci. 56 (7) (2018) 595–603.
[85] C. De Bleye, et al., Critical review of near-infrared spectroscopic methods validations in pharmaceutical applications, J. Pharm. Biomed. Anal. 69 (2012) 125–132.
[86] J.Y. Yhee, et al., Optical imaging of cancer-related proteases using near-infrared fluorescence matrix metalloproteinase-sensitive and cathepsin B-sensitive probes, Theranostics 2 (2) (2012) 179–189.
[87] B.A. Mazin, et al., A superconducting focal plane array for ultraviolet, optical, and near-infrared astrophysics, Opt Express 20 (2) (2012) 1503–1511.
[88] Y. Yang, Application of Near Infrared Spectroscopy Combined with Multivariate Statistical Process Control for In-Line Monitoring of Extraction of Lonicerae Japonicae Flos, Chinese Traditional and Herbal Drugs, 2017, pp. 3497–3504.
[89] H.M. Heyman, J.J.M. Meyer, NMR-based metabolomics as a quality control tool for herbal products, South Afr. J. Bot. 82 (2012) 21–32.
[90] S. Han, et al., Detection of cinnabars in Mongolian medicines using Raman spectroscopy, Guang pu xue yu Guang pu fen xi= Guang pu 35 (10) (2015) 2773–2775.
[91] H. Wikström, et al., On-line content uniformity determination of tablets using low-resolution Raman spectroscopy, Appl. Spectrosc. 60 (6) (2006) 672–681.
[92] K. Yeshi, et al., Geopharmaceuticals of Himalayan Sowa Rigpa medicine: ethnopharmacological uses, mineral diversity, chemical identification and current utilization in Bhutan, J. Ethnopharmacol. 223 (2018) 99–112.
[93] I.G. Tanase, et al., Validation and quality control of an ICP-MS method for the quantification and discrimination of trace metals and application in paper analysis: an overview, Crit. Rev. Anal. Chem. 44 (4) (2014) 311–327.
[94] WHO, WHO Guidelines for Assessing Quality of Herbal Medicines with Reference to Contaminants and Residues, 2007. Geneva.
[95] Y. Wu, et al., Investigation on the bioactivity fingerprint of Qingkailing injection by microcalorimetry, Chin. Pharmaceut. J. 44 (6) (2009) 471–474.
[96] P.D. Hebert, et al., Biological identifications through DNA barcodes, Proc. R. Soc. London, B 270 (1512) (2003) 313–321.
[97] Z. Gao, et al., DNA mini-barcoding: a derived barcoding method for herbal molecular identification, Front. Plant Sci. 10 (2019) 987.
[98] S. Ming, et al., Identification of processed Chinese medicinal materials using DNA mini-barcoding, Chin. J. Nat. Med. 15 (7) (2017) 481–486.
[99] H.J. de Boer, M.C. Ichim, S.G. Newmaster, DNA barcoding and pharmacovigilance of herbal medicines, Drug Saf. 38 (2015) 611–620.
[100] F. Yang, et al., DNA barcoding for the identification and authentication of animal species in traditional medicine, Evid. base Compl. Alternative Med. 2018 (2018).
[101] N. Kane, et al., Ultra-barcoding in cacao (Theobroma spp.; Malvaceae) using whole chloroplast genomes and nuclear ribosomal DNA, Am. J. Bot. 99 (2) (2012) 320–329.
[102] K. Krawczyk, et al., Plastid super-barcodes as a tool for species discrimination in feather grasses (Poaceae: stipa), Sci. Rep. 8 (1) (2018) 1924.