

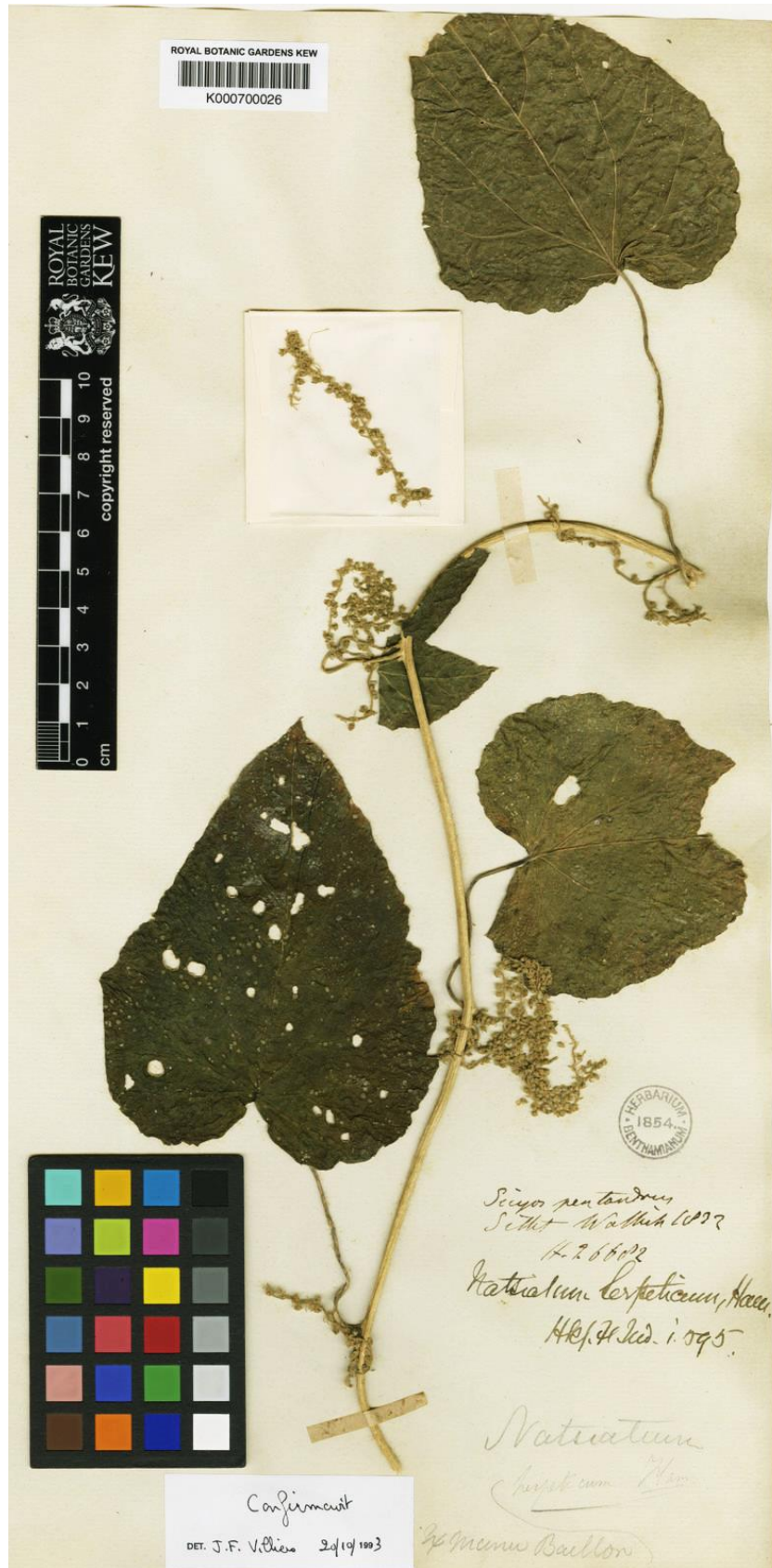
## **CHAPTER 2**

# **LITERATURE REVIEW**

## 2 Literature review

### 2.1 Plant description

*Natsiatum herpeticum* Buch.-Ham. ex Arn. (Icacinaceae family) is a dioecious climbing shrub. It flowers in the months of November-February and fruits in January-February [25]. The acute, serrated, ovate, or sub-orbicular-shaped palmately nerved simple leaves of the *N. herpeticum* plant with an alternate, spiral arrangement are usually covered with stiff white hairs, rendering the leaf rough. The slender petioles (3.5–7.5 cm) of the leaves are yellow-brown crispate pubescent. The leaves of the plant are also characterised by a broadly ovate chartaceous lamina ca. 8–15 x 5–10 cm, which is acute or acuminate at apex, cordate to truncate or abruptly cuneate at base, abaxially prominent and concave adaxially midrib, 3-5 pairs of secondary nerves, sub-parallel tertiary veins, conspicuous reticulate veins, and dentate margins. Pale yellow or greenish, yellow-coloured dioecious pentamerous flowers are arranged in an axillary or supra-axillary hirsute spike-like raceme type of inflorescence. The male flowers have short filaments and sagittate anthers, while the female flowers have subulate staminodes, a hairy ovary, and 2-3 lobed, laterally compressed, ovate, rugose styles. A yellow-green-coloured drupe of 1.5 x 1 cm usually blackens with age. The calyx lobes with valvate aestivation are densely covered with coarse hairs on both the outer and inner surfaces. Linear lanceolate, five-lobed, slender, pendulous, hairy calyx (10–12 cm long), five-membered hairy lanceolate petals, short staminal filaments, pubescent staminodes, sagittate anthers, 2-3 fid style, thickly packed strigose shoots, linear bracts, 2-3 mm long pedicels, strigose young branches, lenticellate old branches, and tuberous roots are a few anatomical and morphological characteristics of the plant (**Fig. 1**) [25–27].



**Fig. 1** A sample herbarium adopted from Plants of the World Online by the Royal Botanic Gardens, Kew; available at <https://powo.science.kew.org/>

### 2.1.1 Taxonomy

Kingdom : Plantae

Division : Angiosperms

Class : Eudicots

Order : Icacinales Tiegh. Ex Tiegh.

Family : Icacinaceae Miers

Genus : *Natsiatum*

Species : *herpeticum*

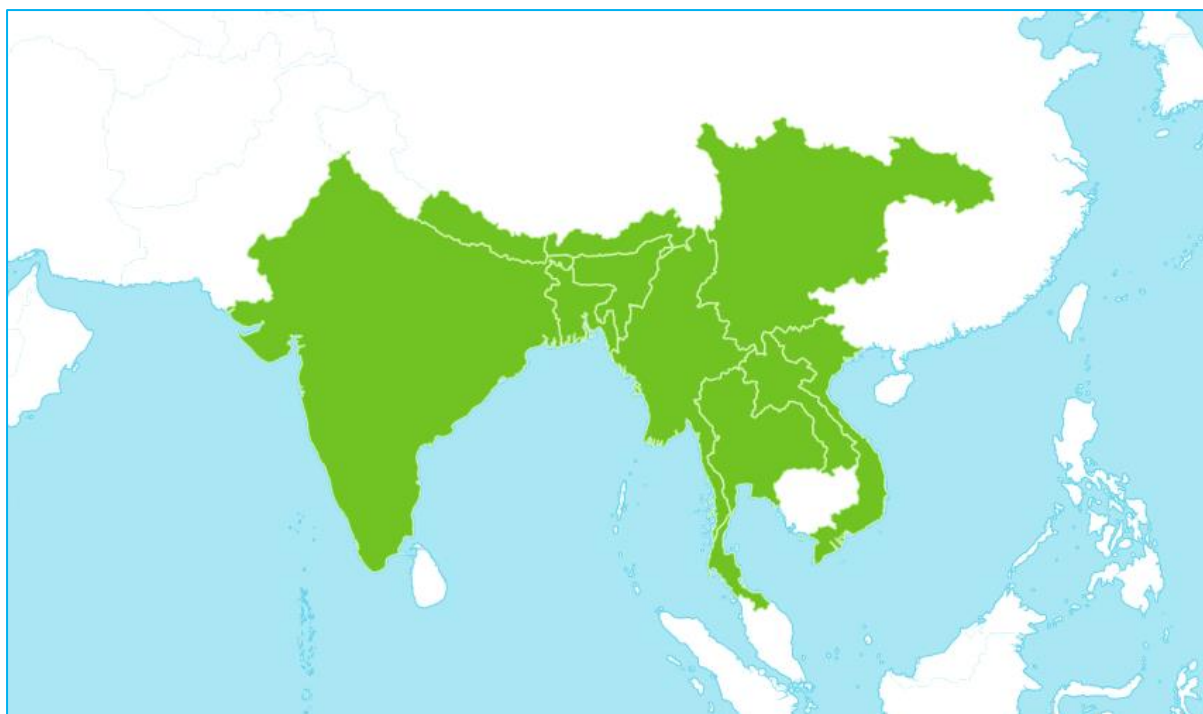
Scientific name : *Natsiatum herpeticum* Buch.-Ham. Ex Arn.

Synonyms : *Natsiatum tonkinense* Gagnep.; *Sicyos pentandrus* Wall.

Common name : Aso-pat (Assamese), au-pat (Assamese), oupat (Assamese), target ruibe (Mishing tribe, Assam)

### 2.1.2 Distribution

The plant grows in scrub jungles and evergreen forests in India, Nepal, Bangladesh (Sylhet, Chittagong), China, Myanmar, Vietnam, and North Thailand. In India, it occurs wildly in rare and particular hilly pockets in Arunachal Pradesh, Assam, Meghalaya, Nagaland, Sikkim, and Tripura (**Fig. 2**) [25, 28].



**Fig. 2** Distribution map of *N. herpeticum* (Adopted from Plants of the World Online by the Royal Botanic Gardens, Kew; available at <https://powo.science.kew.org/>)

## 2.2 Reported phytoconstituents and ethnopharmacology

Basumatary and Narzary [27] reported the phytochemicals, nutritional values, and antioxidant property of six edible plants (including *N. herpeticum*) of Kokrajhar District (BTAD), Assam, consumed mainly by the Bodo community of North-East India. The study revealed the ash content ( $3.42 \pm 0.04$  g/100g fresh sample), crude fat content ( $0.666 \pm 0.01$  g/100g fresh sample), crude protein content ( $5.37 \pm 0.05$  g/100g fresh sample), calorific value ( $54.72 \pm 9.61$  kcal), carbohydrate ( $6.80 \pm 2.40$  g/100g fresh sample), fibre ( $1.99 \pm 0.03$  g/100g fresh sample), and moisture contents ( $83.72 \pm 2.36$  g/100g fresh sample) of the plant. Metal analysis showed the plant to contain sodium ( $92.15 \pm 0.02$  mg/100g dried sample), potassium ( $8436.10 \pm 0.09$  mg/100g dried sample), calcium ( $5.47 \pm 0.02$  mg/100g dried sample), magnesium ( $4.58 \pm 0.03$  mg/100g dried sample), iron ( $2.48 \pm 0.01$  mg/100g dried sample), copper ( $2.10 \pm 0.01$  mg/100g dried sample), zinc ( $0.70 \pm 0.02$  mg/100g dried sample), manganese ( $0.70 \pm 0.01$  mg/100g dried

sample), nickel ( $4.70\pm 0.06$  mg/100g dried sample), chromium ( $0.74\pm 0.09$  mg/100g dried sample), cobalt ( $0.59\pm 0.12$  mg/100g dried sample), selenium ( $0.78\pm 0.41$  mg/100g dried sample), and lead ( $0.75\pm 0.51$  mg/100g dried sample) in the detectable range, while cadmium and arsenic were found to be below detection level. Qualitative phytochemical screening showed the presence of alkaloids, saponins, cardiac glycosides, steroids, coumarins, phenols, tannins, flavonoids, anthocyanin, phlobatanins, carbohydrates, proteins, and lignin, whereas anthraquinones were found to be absent. Apart from these, the total phenolic content, flavonoid content, and vitamin C content were reported to be  $10.45\pm 0.68$  mg gallic acid equivalents/g dried extract,  $0.29\pm 0.11$  mg quercetin equivalents/g dried extract, and  $85.71\pm 5.71$  mg/100g fresh weight, respectively. The antioxidant activity of a methanolic extract of *N. herpeticum* was also reported by the same group. The methanolic extract showed IC<sub>50</sub> values of  $1658.47\pm 2.72$  µg/ml,  $283.23\pm 0.49$  µg/ml, and  $38.54\pm 0.19$  µg/ml in the DPPH, ABTS, and H<sub>2</sub>O<sub>2</sub> assays, respectively. While the IC<sub>50</sub> values of ascorbic acid as the standard were found to be  $25.01\pm 0.52$  µg/ml,  $73.67\pm 0.74$  µg/ml, and  $19.02\pm 0.01$  µg/ml, respectively, in each method [27].

Another study reported *N. herpeticum* to contain camptothecin (an anticancer alkaloid) based on HPLC analysis of different species of the Icacinaceae family. The plant was reported to contain the potent anticancer molecule camptothecin in the dried fruits (0.026%) and in fresh twigs ( $0.0029\pm 0.0024\%$ ) [29].

Ethnopharmacology is an integrative and holistic approach to drug discovery that includes the observation, description, and investigation of the biological activity of traditional remedies. When ethnopharmacological uses are considered, the significant impact of medicinal plants becomes evident. Though not much literature is available about the plant, the leaves and tender shoots of *N. herpeticum* have been reported to be used by the Mishing (India) tribe [30] and Bankariya (Nepal) [31] communities as part of their delicacies. Also, this plant is consumed as

a part of the custom of eating rare herbs during Bohag Bihu (a widely celebrated festival in Assam, India) by various ethnic groups [22]. Literature suggests that the plant is used by the Sonowal Kacharis (a tribe in north-east India) for the treatment of constipation and stomach pain as a practice of a traditional system of medicine [32]. The leaf paste of the plant is also used for quick healing of cuts and wounds [22].

### **2.3 Network Pharmacology**

Limitations of the one-drug/one-target/one-therapeutic approach (the concept of the "magic bullet") and expansions of computational power and omics technologies triggered the emergence of network pharmacology, a new paradigm in drug discovery dedicated to the understanding of drug actions and interactions with multiple targets [33]. This system biology and polypharmacology based multidisciplinary concept purveying a novel network mode of "multiple targets, multiple effects, complex diseases" with a rewarding abetment to prognosticate the off-target effects at a higher efficiency replaced the "magic bullets" by "magic shotguns" [34]. The interactions of multiple genes and functional proteins, and impairment of a complete regulatory network are the genesis of complex diseases like cancer, diabetes, etc. Henceforth, understanding the entire biological network underlying a disease is a must to achieve the objective of successful diagnosis and treatment of complicated disorders. In 2007, Hopkins introduced network pharmacology as an innovative approach to answering questions pertaining to the manner in which a target inhibits or activates the disease phenotypes within the disease network [34, 35]. According to network pharmacology, disease mechanisms are networks that can be effectively treated with a combination of multiple synergistic drugs. By addressing the disease network at the system level through synergistic and deadly interactions, this ideally results in therapies that are less susceptible to drug resistance and have fewer adverse effects. Recently, this integrative in silico approach has steadily started to gain

popularity as a way to better elucidate the processes underlying the synergistic therapeutic effects of traditional medicines [34, 35].

Unsustainable use and traditional collection and application methods of medicinal plants, the source of cost-effective and natural remedies, have resulted in the depletion of several plant species. Reengineering traditional medicines guided by holistic philosophies and extensive experimentation in multicomponent treatments may be a promising lead for preliminary screening of medicinal plants' bioactive compounds and thereby, controlling the complicated nature of diseases. Incorporating network pharmacology into traditional medicine will therefore provide distinctive and innovative possibilities for identifying active components, biomarkers, and the scientific underpinnings of traditional medicine based on the intricate biological systems of the human body [35].

Network pharmacology can have an impact on the drug development process' two primary techniques. One is to create a practical network model and anticipate the drug target using data from open databases or previously published studies. The network equilibrium principle should then be examined through the functional drug's mechanism [34]. Gu et al. assessed the impact of Rheidin A, C, and Sennoside C using this methodology, and this was the first publication on multiple component drugs for type II diabetes [36]. The second strategy involves combining bioinformatics techniques with high-throughput screen (HTS) technology to reconstruct a "drug target disease" network prediction model. In this method, the interaction between the drug and the model was compared in order to analyse the mechanism of drugs in the biological network. Li et al. discovered that multilayer networks may underlie the combined processes of herbal formulas and used the Liuwei Dihuang pill (a Chinese herbal medicine formula) to anticipate the appropriate network targets in disease treatment [37]. Salvianolic acid B was shown to be appropriate and practical for the treatment of cardiovascular diseases by combining the aforementioned approaches [38]. Regulation of the signalling pathway with multiple

channels, improvement of the efficacy of drugs, decrease in adverse effects, increase in clinical trial success rate, and reduction in drug discovery expenses are a few benefits of the application of the concept of network pharmacology [39].

Shen et al. predicted the effect of *Sanguisorba officinalis* L., a traditional Chinese medicine, against *Helicobacter pylori* infection using a network pharmacology analysis approach. This analysis revealed eight active compounds in *S. officinalis*. 49 targets were identified as possible targets for the therapy of *H. pylori* infection out of 222 putative targets of *S. officinalis*. And these 49 targets were significantly enriched in KEGG pathways like pathways in cancer, microRNAs in cancer, and the TNF signalling pathway, as well as GO processes like protein kinase B signalling, protein kinase activity, and protein kinase binding. Five main targets (AKT1, VEGFA, EGFR, SRC, and CCND1) were identified using interaction analysis and confirmed via molecular docking and RT-qPCR [40]. Only 4 out of 29 components in *Caesalpinia pulcherima* were found to be active and considered for interaction studies. Protein-protein interaction data is suggestive of the significant interactions of ESR-1, ESR-2, ESRRA, MET, VEGF, FGF, PI3K, PDK-1, MAPK, PLK-1, NEK-2, and GRK in the network, which were further investigated by in silico and in vitro studies [41].

## **2.4 Pharmacological studies**

### **2.4.1 Antibacterial activity**

The omnipresence of bacteria intensifies bacterial infections as a threat to mankind. The susceptibility to infection is high owing to the ability of the causative agent to destroy the host's cells or the response of the host immune system towards pathogenic agents. An array of transmitters and modes of transmission are opportunistic advantages that promote the pathogenicity and infectivity of the microorganism [42]. Even though the success of antibiotics is commemorated, the undersupply of essential antibiotics in many low- and middle-income

countries and the upshot of antimicrobial resistance (AMR) are jeopardising the constructive effects of antibiotics on health. Skyrocketing AMR, a global public health threat declared by the World Health Organization (WHO) and a societal issue, might slow down the achievement of the Sustainable Development Goals (SDGs) [43]. A dire trend was confirmed by the first of its kind intercontinental gauging of AMR burden in 2019, which in the same year approximated 4.95 million fatalities related to AMR and 1.27 million deaths directly attributable to AMR. A predicted worldwide mortality rate of 10 million per year by 2050 and weighty economic switchovers are the aftermath of AMR [44]. The search for effective antimicrobials continues, as its shortfall will menace the successful performance of common surgical procedures and the treatment of other medical conditions with modern medicine.

Since ages, natural products have laid the foundation stone for the development of promising antimicrobials like  $\beta$ -lactams, macrolides, tetracyclines, aminoglycosides, lincosamides, and glycopeptides. Of the nature-based products against drug resistant bacteria from variegated terrestrial sources, 46% were of plant origin. These products exerted their actions through different mechanisms controlling multi-resistant pathogens. Natural antimicrobial agents are renowned for having non-toxic properties and a variety of chemical scaffolds. Importantly, it cannot be denied that natural compounds have sparked a lot of interest in supplanting the effectiveness of ineffective antibiotics [45].

#### **2.4.2 Anti-inflammatory activity**

Due to its complex pathogenesis, inflammation is a crucial factor in the development and progression of numerous diseases and their complications. Inflammation is a series of protective immune responses triggered by injury factors, such as pathogens, damaged cells, and other stimuli. It regulates numerous physiological and pathological processes in the body by influencing various cells and microenvironmental factors. Inflammation elicits a response

from tissue stromal cells and immune cells, permitting the entrance of cells and proteins from the vascular system into damaged or infected tissues, thereby promoting repair. Despite the fact that the occurrence of self-limiting inflammation is physiological and essential for eliminating pathogens, the persistence of inflammation is detrimental to the systemic reactions of the afflicted organs and other organs. According to multiple studies, inflammation regulates the development and progression of numerous complex diseases. Several anti-inflammatory drugs, including non-steroidal anti-inflammatory drugs (NSAIDs) and steroidal anti-inflammatory drugs, have been detected in the market. However, the medications have a few drawbacks due to the fact that their long-term use causes adverse reactions in numerous organs [46]. Zhu et al. evaluated the efficacy of *Andrographis paniculata* (Burm.f.) Nees, a formulated Traditional Chinese Medicine in inflammatory response using LPS-induced NO production in RAW 264.7 cell line, Western blot and RT-PCR. It was indicated that it suppressed the production of inflammatory mediators and exerted anti-inflammatory effects involving pathways in cancer, inflammation, and immunological processes [46]. The ethanolic leaf extract of *Artemisia annua* L., enriched with artemisinin and anthocyanins, exhibited an anti-inflammatory effect in human neuroblastoma cells (SH-SY5Y) treated with lipopolysaccharide (LPS) by reducing TNF- $\alpha$  mRNA gene expression [47]. The standardised *Aronia melanocarpa* dry extracts with 25% anthocyanins reduce the level of inflammatory markers (IL-1 $\beta$ , TNF- $\alpha$ , MDA) in lipopolysaccharide-stimulated RAW 264 cells [48]. The anti-inflammatory effect of fruit and seed extracts of *Elettaria cardamomum* in periodontal infections was also evidenced from inhibition of the NF- $\kappa$ B signalling pathway and decreased levels of IL-1 $\beta$ , IL-8 and TNF- $\alpha$  by LPS-stimulated macrophages [49].