EXTENDED ABSTRACT

Quality Control studies and ethnopharmacological evaluation of *Natsiatum herpeticum* Buch.-Ham. ex Arn.

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Background

Harnessing nature in search of remedies for an array of diseases has been an old practice. Folkloristic use of flora, fauna, and mineral sources for the treatment of a range of ailments and diseases paved the way for the discovery of natural products or their derivatives. Since 1980, approximately 51% of approved drugs have been derived from natural products. By 2050, the international herbal market is predicted to generate a turnover of $5 trillion. However, only 15% of the higher plants have been screened for phytoconstituents and about 6% for their pharmacological effectiveness. Meanwhile, emerging risks to human health necessitate a concerted effort to find both preventive and therapeutic approaches, with natural products at the centre of initiatives to develop novel treatments and lower disease transmission and associated mortality. Despite various bottlenecks, natural product research has seen a remarkable renaissance in the past few years. The advent of robust modern analytical technologies, including chemotaxonomic studies, chemical fingerprinting, dereplication techniques, bioinformatics, chemoinformatics, and metabolomics, has made significant contributions to NP-based drug discovery in recent times. Natural product research has gradually embraced computational approaches involving artificial intelligence and machine learning algorithms. This technique utilising in silico data is quickly gaining popularity due to its low cost and comparable predictability. Consequently, network analysis has multiple applications and promising prospects in relation to the drug discovery and development process. Network pharmacology analysis not only introduces novel therapeutic options, but also seeks to enhance the safety and efficacy of existing drugs.

Natsiatum herpeticum Buch.-Ham. ex Arn. is a very less exploited shrub found in evergreen forests and scrub jungles of South Asia and Southeast Asian countries. It is being considered a wild edible plant by the Bankariya community of Nepal, and the Mishing, Sonowal Kachari, and several ethnic groups in the north-east region of India. Despite several

ethnopharmacological claims, the phytochemical constituents and plausible medicinal properties of *N. herpeticum* are yet to be investigated. The findings from the literature review reaffirm the fact that, in spite of the remarkable advantageous utility of the plants in the treatment of a range of ailments and disorders, authenticated documentation and scientific validation of the same for the preservation of biocultural diversity have not been done yet. A deeper insight into the pharmacological and toxicological aspects is also awaited, which, whenever elucidated, will fruitfully lead to the discovery of promising new therapeutic agents.

The research work presented here addresses four major objectives to fill the lacunae in the present knowledge. The first objective of the study is to validate the pharmacognostical quality control assessment of *N. herpeticum* in accordance with standard guidelines. The second objective is to determine the toxicity profile of the plant. The third objective includes qualitative screening of the aqueous plant extract and network pharmacology-based prediction of the antibacterial and anti-inflammatory potential of the detected compounds. The fourth objective deals with the experimental validation of the pharmacological activity of the aqueous extract of *N. herpeticum* using in silico-in vitro-in vivo approach.

Material and methods

Plant material was authenticated using a classical taxonomical approach and the DNA barcoding method. The quality control assessments were performed as per the World Health Organization (WHO) guidelines to ensure the purity and quality of the plant material. In order to determine the toxicity profile, an acute toxicity study and a repeated dose 28-day oral toxicity study were performed as per OECD guidelines. Aqueous extract of the plant was subjected to QToF-MS analysis to analyse the phytocompounds. Network pharmacology prediction was made for the detected compounds with drug-likeness properties for their

potential compound gene-disease gene interactions in bacterial infections and inflammation. In order to validate the antibacterial potential predicted by network pharmacology, in vitro studies followed by molecular docking study and molecular dynamic simulations were performed. Similarly, the anti-inflammatory potential of the extract was validated using an in vitro-in vivo approach.

Results and discussion

The plant was identified by traditional identification method and DNA barcoding analysis (GenBank accession number: OP121185). The quality control assessment ensured the quality and purity of the collected plant materials. Acute toxicity study revealed no signs of toxicity after administration of single dose of 5000 mg/kg in the animals. However, in the repeated dose 28-day oral toxicity study, alterations in food and water intake, and biochemical parameters were seen in both male and female groups. Two female animals displayed leukocyte infiltration and sinusoids dilatation, which were consistent with the changes in biochemical parameters. Thus, it was anticipated that repeated exposure to a high dose (2000 mg/kg) aqueous extract of *N. herpeticum* may possess deleterious effects, particularly in hepatic tissues. 21 representative compounds were detected by QToF-MS analysis, of which 14 compounds were found to possess drug-likeness property. These compounds, when subjected to network pharmacology predicted different pathways by which the compounds may exhibit antibacterial and anti-inflammatory activity. Network pharmacology showed TNF and IRAK4 to be the two gene targets that can be modulated by the compounds to exert antibacterial activity. Analysis of the compounds with drug-likeness properties using molecular docking (against 1KZN, 2VF5, 2W9S, and 4CJN) and MD simulation suggested compound HME (ΔG binding energy of -37.7 Kcal/mol and docking scores of -4.75, -7.53, -6.15, and -5 against 1KZN, 2VF5, 2W9S, and 4CJN, respectively) to be the potent

compounds against these bacterial targets. Similarly, the aqueous extract of *N. herpeticum* exhibited potential antibacterial activity (bacteriostatic) against *E. coli* and *P. aeruginosa* with an MIC of 50 μg/ml in microtiter-plate dilution assay. However, the extract at all concentrations failed to inhibit Gram-positive bacteria in the microtiter-plate dilution assay, demonstrating a selective inhibition of Gram-negative bacteria. Overall, these data suggest that *N. herpeticum* not only exhibits potential bacteriostatic activity against Gram-negative bacteria but can also modulate host-immune responses via TNF and IRAK4 associated pathways.

Similarly, analysis of interactions between 14 compound-associated genes and inflammation-linked genes revealed that the plant constituents may modulate TNF, PTGS2, EGFR, STAT3, PPARG, PTGER4, PPARA, NOS2, TRPV1, and JAK2 associated pathways to induce an anti-inflammatory response. This finding was consistent with the in vitro studies that demonstrated plant extract to possess a good anti-inflammatory effect with IC_{50} values of 98.76, 85.73, and 96.16 μg/ml in protein denaturation, proteinase inhibition, and haemolysis inhibition assays, respectively. In support of this, the extract also showed dose- and time-dependent anti-inflammatory effects in vivo, as shown by a decrease in paw swelling volume measured in carrageenan induced paw-oedema method.

Conclusion

Plants have long been a well-known treasure trove of potential bioactive natural products. Though the thrust for novel compound discovery continues, the geographical distribution of some plant species (like *N. herpeticum* found in only a few South and Southeast Asian countries) remains a challenge to such exploitation. As repeated administration of a high dose exhibited signs of liver toxicity, a chronic toxicity study is required to ensure the toxic potential of this edible plant. Further analysis of the extract and compound HME against other bacterial strains will provide more insight into the findings. As inflammation is associated with a myriad of diseases or conditions, studies emphasising particular diseases or conditions will aid in understanding the possibility of the clinical utility of the extract and its components.