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Original Article

Phytochemical Screening, Acute Toxicity Study, and Pharmacological Evaluation of *Natsiatum herpeticum* Buch.Ham. ex Arn.

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ABSTRACT

Background: Plants are an open treasure of diverse pharmacologically active scaffolds. *Natsiatum herpeticum*, a least exploited plant, is known to be consumed by various ethnic groups of Asian origin as edible or as part of traditional remedy. **Materials and Methods:** The Ethanolic Extract of *N. herpeticum* (EENH) was evaluated for acute oral toxicity using acute toxic class method (OECD 423). Here, we aimed to investigate the free radical scavenging (antioxidant) and *in vitro* anti-inflammatory property of the plant extract. **Results:** Acute toxicity result suggests the plant to be safe and can be categorised under category 5 of GHS classification. The EENH showed promising antioxidant activity in DPPH (IC_{50} value= 84.1 µg/mL) and ABTS (IC_{50} value=81.8 µg/mL) method. Similarly, the plant extract demonstrated good *in vitro* anti-inflammatory activity (IC_{50} value=80.88-93.26 µg/mL). **Conclusion:** The plant can be considered as safe to be consumed. The plant extract exhibited promising *in vitro* antioxidant and anti-inflammatory activity, which are further to be tested in *in vivo* models.

Keywords: Acute toxicity, Antioxidant, Anti-inflammatory, *Natsiatum herpeticum*.

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INTRODUCTION

For a significant number of decades, natural products, especially plant-derived products, have been considered as treasure of potential therapeutics. Given the diverse chemical space, complex nature, and desirable physicochemical properties, the natural products have been considered as the source of either lead molecules or indirectly as privileged scaffolds for half of all synthetic small molecules.[1,2] Natural products are regarded as the centre of initiatives for development of both novel safer therapeutics and preventive medications against the emerging risks to human health.[3] Despite the potential of natural products, they also present themselves with bottlenecks in the drug discovery process, including technical difficulties in compound extraction and isolation, and their characterization and optimization. These disadvantages led to the waning interest in natural product development by the pharmaceutical companies in the past few decades.[4] Optimistically, though there exist ample drawbacks, science must move on and must focus on the betterment of human health. Nevertheless, many medicinal plants are yet to be explored for their phytoconstituents and their potential therapeutic implications. On the other hand, limited global distributions also can be regarded as one of the reasons of lesser emphasis from the scientific community towards certain plants. *Natsiatum herpeticum* Buch.Ham. ex Arn. is a very less exploited dioecious shrub found in evergreen forests and scrub jungles of India, China, Bangladesh, Nepal, Vietnam, Myanmar, and North Thailand. However, there exist lacunae in the present knowledge about the experimental validation of the traditional claims as available in the literature. It is reported that the tender shoots and leaves have been used by the Mishing tribe of India[5] and Bankariya community of Nepal[6] as a delicacy. Different ethnic groups of Assam (India) consume this plant as an ancestry of Bohag Bihu (a most celebrated festival) custom of eating rare herbs.[7] This plant is reported to be used by Sonowal Kacharis (a north-east Indian tribe) as a traditional remedy against constipation and stomach pain.[8] Apart from this, leaf paste of *N. herpeticum* is also known for its medicinal properties against inflammation and pain-associated conditions including backache,[9] cuts and wounds,[7] and headache.[10] Additionally, literature suggests *N. herpeticum* to possess antioxidant property.[11] Interestingly, the fruit (0.026%) of this plant was found to contain camptothecin

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(a potent anti-cancer molecule).[12] Despite this the phytochemical constituents and plausible medicinal properties of *N. herpeticum* are yet to be investigated. We anticipate this lacuna in the current research to be associated with limited plant distribution (available in a few Asian countries only). Nonetheless, though the plant is being consumed as a wild edible herb by various communities of different regions, the dearth of information on toxicity profile and possible pharmacological activities persuaded us to undertake this study to fulfil the knowledge gap. In continuation to our previous efforts to validate ethnopharmacological claims of less common plants, we aimed to validate the acute toxicity profile of ethanolic extract of *N. herpeticum* by performing acute oral toxicity study as per the OECD-423 guideline (acute toxic class method). Also, we reported the *in vitro* antioxidant and anti-inflammatory properties of Ethanolic Extract of *N. herpeticum* (EENH).

MATERIALS AND METHODS

Authentication of plant material

The collected plant material of *N. herpeticum* was identified and authenticated through morphological and molecular taxonomic methods. The taxonomical surveillance was carried out at North East Herbarium of Ayurveda Research (NEHAR), Central Ayurveda Research Institute (CARI), Guwahati, Assam, India and Department of Life Sciences, Dibrugarh University, Assam, and confirmed the gathered plant to be *Natsiatum herpeticum* Buch.-Ham. ex Arn. Ribulose-1, 5-bisphosphate carboxylase/oxygenase large subunit (rbcL) and maturase K (matK), the molecular markers were sequenced, and sequence homology analysis was done using Basic Local Alignment Search Tool (BLAST). The BLAST analysis revealed 100% and 97.16% identity to rbcL (RID: 7WB85MFP01N) and matK (RID: 7WBJCY5K013), respectively.

Extraction and phytochemical screening

The shade dried aerial portions of the plant *N. herpeticum* were mechanically pulverized into coarse powder, and then extracted using ethanol. Upon elimination of the traces of solvent used, the extracts were concentrated and placed in desiccators at that point of subjection to preliminary screening. Preliminary phytochemical analysis of the prepared extract was conducted to assort the presence of various classes of phytochemicals.

Animal selection and housing

The experiments were carried out as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. The protocol was approved by Institutional Animal Ethics Committee or IAEC vide Dean/2019/IAEC/1644 on 17th November 2019. Non-pregnant and nulliparous female laboratory Wistar rats (8-12 weeks old and weighing 150±20 g) were obtained from the Central Animal House of Banaras Hindu University, Varanasi, India. Rats were kept for acclimatization in the departmental animal house for a week with artificial lighting maintaining 12 hr day-night cycle with temperature of 22 ± 3°C (relative humidity= 40-60%). The animals were grouped and kept in polypropylene cages provided with free access to laboratory diets and water *ad libitum*.

Acute oral toxicity study

The acute toxic class method was conducted to determine the acute oral toxicity study in compliance with the Organization for Economic Cooperation and Development (OECD) guideline for testing of chemicals (OECD guideline 423) and Good Laboratory Practice (GLP).[13] The rats were divided into two groups using computer-generated randomization tables. Each group consisted of three animals: the control group and the animals that received Ethanolic Extract of *Natsiatum herpeticum* (EENH) at a dose of 5000 mg/kg body weight. Following dose administration, the rats were carefully observed during the first 30 min and with special attention during initial 4 hr. Then the rats were individually monitored daily for 14 days and after completion of the study, blood of the rats was collected for haematological and biochemical assessment. The animals were sacrificed for necropsy and histopathological analyses at the end of day fourteen.

In vitro antioxidant Assay

DPPH free radical scavenging method

The radical scavenging activity of EENH was determined using freshly prepared methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent as described by Gashaye and Birhan 2023.[14] Ascorbic acid was used as the standard. 1M DPPH solution was added to samples of extract and standard. Post incubation in the dark for about 30 min, absorbance of the solution was recorded at 517 nm.[14] The % DPPH radical scavenging activity (in triplicates) was calculated as mentioned below.

$$\% \text{ DPPH radical scavenging} = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100$$

where A_{sample} was absorbance of the sample suspended in reagent solution and $A_{control}$ was absorbance of the reagent control after 30 min of incubation.

ABTS radical scavenging method

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation decolorization assay was performed to evaluate the free radical scavenging property of EENH using ascorbic acid as standard. ABTS solution was prepared by adding 10 mg ABTS with 2 mg potassium persulfate in distilled water and kept aside for 12-16 hr in dark place prior to its use. 1 mL of the solution was diluted with 60 mL of methanol. Varying concentrations of EENH and ascorbic acid (20-100 µg/mL) are incubated with the ABTS solution. The absorbance was measured at 734 nm

and percentage inhibition was calculated in triplicates using the following formula.[15]

$$\% \text{ ABTS radical scavenging} = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100$$

where A_{sample} was absorbance of the sample and $A_{control}$ was absorbance of the control.

In vitro anti-inflammatory assays

Proteinase inhibition

Test sample (1 mL) containing EENH (0.02 mL) and ethanol (0.98 mL) was mixed with 20 mM tris-HCl buffer of pH 7.4 (1 mL) and trypsin (0.06 mg) to obtain the reaction mixture. The mixture was incubated for 5 min at 37°C. Following this, the reaction mixture was incubated for another 20 min after addition of 0.8% w/v casein (1 mL). The incubated mixture was added with 70% perchloric acid (2 mL) and subjected to centrifuge. The supernatant was used to obtain the absorbance at 210 nm against buffer solution (blank).[16,17] The percentage proteinase inhibition and IC_{50} were determined.

Protein denaturation method

The EENH (0.02 mL) was mixed with 1% bovine albumin (0.2 mL) and phosphate buffer saline of pH 6.4 (4.78 mL) to get a desired reaction mixture. The obtained mixture was incubated for 15 min at 37°C. The same mixture was then kept for 5 min at 70°C followed by cooling. The absorbance of the sample was measured at 660 nm.[17] The percentage inhibition and IC_{50} were determined.

Membrane stability

The whole blood obtained from healthy human volunteer was subjected for centrifugation at 3000 rpm (5 min), followed by triple washing with equal volume of 0.9% NaCl (normal saline). After measuring the blood volume, it was reconstituted with 10 mM sodium phosphate buffer of pH 7.4 as a 10% *v/v* suspension.[16] The EENH (0.05 mL) was added with obtained blood cell suspension (0.05 mL) and phosphate buffer (pH=7.4; 2.95 mL), and this sample solution was incubated for 20 min at 54°C followed by centrifugation at 2500 rpm (3 min). The absorbance of the supernatant was measured at 540 nm using phosphate buffer as control. The percentage inhibition and IC_{50} were determined.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software, USA). All the data were determined as mean ± standard deviation and p value < 0.05 represents statistical significance. Relative organ weight, haematological, and biochemical parameters were analyzed using one-way ANOVA (Dunnet's multiple comparison test).

RESULTS AND DISCUSSION

Extraction and phytochemical screening

The percentage yield of EENH was determined to be 6.0921%w/w. The qualitative phytochemical screening of the extract indicated the presence of secondary metabolites like alkaloids, glycosides, phenolic compounds, terpenoids, and steroids (Table 1).

Acute toxicity study

All the animals receiving 5000 mg/kg EENH showed no behavioral alterations during the experimental period of 14 days There were no evident signs of physical or clinical abnormalities. Both the groups, i.e., control and EENH group exhibited weight gain by day 14 that indicated the normal animal growth during the experiment. After the experiment was over, on day 14 necropsy revealed no significant changes in the organ and relative organ weights (Table 2). Estimation of haematological and biochemical parameters revealed comparative profile among the control and EENH group (Tables 3 and 4). Except for white blood cell count (EENH vs control: 18.66 ± 0.66 vs 14.17 ± 2.12 × 10^9/ L) and alkaline phosphatase (175.2 ± 2.50 vs 166.2 ± 2.14), all other estimated parameters demonstrated insignificant changes. Necropsy study and histopathological evaluation showed no observable changes or lesions in the animal organs, especially, heart, lungs, liver, and kidney (Figure 1). Considering this, EENH can be classified under category 5 as per GHS classification.

In vitro antioxidant activity

Free radical scavenging activity of EENH using DPPH and ABTS method were evaluated. The control (Ascorbic Acid) and EENH

Table 1: Preliminary phytochemical screening of *Natsiatum herpeticum*.

Phytoconstituent	Name test/ Reagent	Observation
Alkaloids	Dragendorff's reagent	+
Anthraquinone glycosides	Borntrager's test	–
Cardiac glycosides	Keller-Killiani's test	+
Coumarin glycosides	-----	+
Saponin glycosides	Froth formation test	+
Tannins	Ferric chloride test	+
Flavonoids	Alkaline reagent test	+
Steroids and Triterpenoids	Salkowski test	+

+ indicates detected; - indicates not detected.

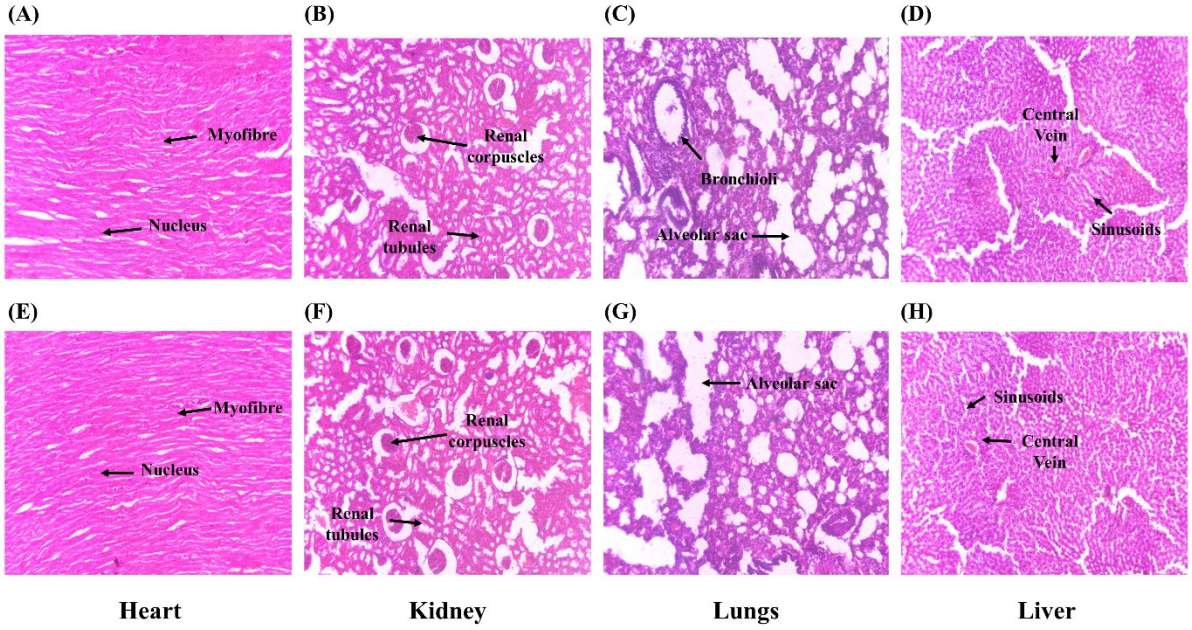

Figure 1: Representative histological observations in control and EENH treated animals with no signs of toxicity.

Table 2: Relative Organ Weight (ROW) after day 14. All values are Mean ± SD (*n* = 3). **p*<0.05 as compared to control [One-way ANOVA; Dunnet's test).

ROW (%)	Liver	Heart	Kidney	Spleen	Stomach	Pancreas	Lung	Ovary
Control	3.36 ± 0.13	0.35 ± 0.11	0.81 ± 0.08	0.21 ± 0.03	0.89 ± 0.06	0.17 ± 0.02	0.58 ± 0.05	0.29 ± 0.07
EENH	3.36 ± 0.12	0.36 ± 0.16	0.83 ± 0.08	0.22 ± 0.08	0.90 ± 0.02	0.17 ± 0.05	0.59 ± 0.07	0.30 ± 0.06

Table 3: Changes in haematological parameters after day 14. All values are Mean ± SD (*n* = 3). **p*<0.05 as compared to control [One-way ANOVA; Dunnet's test).

Haematological Parameters	Control	EENH
RBC ($\times 10^6$/ mm^3)	6.36 ± 0.50	6.44 ± 0.32
WBC ($\times 10^9$/ L)	14.17 ± 2.12	18.66 ± 0.66*
Platelet ($\times 10^3$/ mm^3)	770 ± 32.12	758 ± 43.18
Haemoglobin: g/dL	12.34 ± 0.62	12.88 ± 0.56
Mean Corpuscular Volume (MCV); fL	60.5 ± 1.78	60.78 ± 0.83
Packed Cell Volume (PCV); %	43.2 ± 0.98	42.2 ± 1.24
Mean Corpuscular Haemoglobin Concentration (MCHC); g/dL	31.8 ± 0.87	30.9 ± 1.00

Table 4: Effect of single 5000 mg/kg dose EENH on biochemical parameters. All values are Mean ± SD (*n* = 3). **p*<0.05 as compared to control [One-way ANOVA; Dunnet's test).

Biochemical Parameters	Control	EENH
Total protein (g/L)	59.4 ± 2.58	60.2 ± 2.03
Albumin (g/L)	39.2 ± 1.88	41.6 ± 2.10
Globulin (g/L)	17.9 ± 1.22	20.6 ± 1.56
Aspartate Aminotransferase (AST) (U/L)	129 ± 1.68	134 ± 4.06
Alanine Aminotransferase (ALT) (U/L)	77.2 ± 3.6	80.8 ± 6.10
Alkaline Phosphatase (ALP) (U/L)	166.2 ± 2.14	175.2 ± 2.50*
Triglycerides (mg/dL)	39.8 ± 6.50	40.2 ± 3.88
Total cholesterol (mg/dL)	66.8 ± 6.87	69.00 ± 2.80
Creatinine (mg/dL)	0.59 ± 0.08	0.58 ± 0.14

extract showed concentration dependent free radical scavenging in both DPPH and ABTS method. The organic compound DPPH consists of stable free radical molecules and appears as a dark purple substance that becomes transparent or yellowish when reduced by an antioxidant.[15,18] The IC_{50} value is defined as the crude extract concentration that inhibits the oxidation process by 50%. It is inversely proportional to antioxidant capacity, and a lower IC_{50} value indicates more effective antioxidant activity. The EENH showed the free radical scavenging activity within the range of 18.3±1.2 to 56.4±1.4% at the concentration range of

Table 5: ***In vitro*** **antioxidant activity of Ethanolic Extract of** ***N. herpeticum*** **(EENH).**

Concentration (µg/mL)	% DPPH radical scavenging		% ABTS radical scavenging	
	Control	EENH	Control	EENH
20	40.6±1.2	18.3±1.2	42.6±1.2	16.3±1.5
40	48.4±1.8	27.4±1.5	50.4±0.9	29.4±1.5
60	56.8±1.8	37.4±1.2	58.5±1.8	39.4±0.3
80	68.0±1.5	50.6±0.6	69.7±1.5	49.6±0.3
100	80.8±1.2	56.4±1.4	83.6±0.9	58.4±0.6

*Control: Ascorbic acid.

Table 6: **Anti-inflammatory potential of Ethanolic Extract of** ***N. herpeticum*** **(EENH).**

Concentration (µg/mL)	% inhibition of proteinase (Proteinase inhibition assay)		% inhibition of protein denaturation (Protein denaturation assay)		% haemolysis inhibition (Membrane stability assay)	
	Control	EENH	Control	EENH	Control	EENH
20	22.3±0.1	18.3±0.1	20.2±0.8	18.2±0.5	22.4±0.8	16.6±0.9
40	28.6±1.8	26.8±0.1	30.6±1.0	27.0±0.6	34.3±0.5	24.3±0.1
60	39.8±1.5	38.2±0.7	41.8±0.8	32.4±0.3	48.5±0.5	35.4±0.5
80	53.3±1.2	47.2±0.4	53.2±0.8	41.2±0.2	59.4±0.7	42.2±0.4
100	68.7±0.8	62.6±0.2	70.5±0.5	56.2±0.5	76.4±0.9	56.0±1.0

*[% inhibition = 100 x (1-A2/A1); where, A1 = absorbance of the control, A2 = absorbance of the EENH]; Control- Aspirin.

20-100 µg/mL in DPPH method, with an IC_{50} value of 84.1 µg/mL as compared to IC_{50} value of 42.16 µg/mL of control (Table 5). In ABTS method, the scavenging activity of EENH was measured at the concentration range of 20-100 µg/mL (Table 5). In the presence of potassium persulfate, ABTS cation produces a dark blue-green solution that turns light green when reduced by hydrogen-donating antioxidants.[15] The maximum concentration of EENH showed the highest inhibition of 58.4±0.6% as compared to 83.6±0.9% of control. The IC_{50} value was determined to be 81.8 µg/mL as compared to 38.36 µg/mL of control. These results indicate that *N. herpeticum* could be a potential source of natural antioxidants. Our result also supports the previous finding of Basumatary and Narzary,[11] which reported the antioxidant property of *N. herpeticum* in different methods. This property may be attributed to the higher Vitamin C (an antioxidant) content (85.71±5.710 mg/100g of fresh weight) found in the plant.[11]

In vitro anti-inflammatory activity

In proteinase inhibition assay, EENH demonstrated inhibition of proteinase in a concentration dependent manner (Table 6). The EENH mediated percentage inhibition of proteinase was between 18.3±0.1 to 62.6±0.2% when used at concentration range of 20-100 µg/mL. The IC_{50} value of EENH was found to be 80.88 µg/mL as compared to 72.70 µg/mL of control (aspirin). In protein denaturation assay, EENH demonstrated percentage inhibition within 18.2±0.5 to 56.2±0.5% as compared to control group inhibitory percentage ranging from 20.2±0.8 to 70.5±0.5 (Table 6). The IC_{50} value of EENH was calculated to be 93.26 µg/mL (control IC_{50}= 70.94 µg/mL). The inhibitory effect of EENH in membrane stability assay is shown in Table 6. A concentration of 91.23 µg/mL was recorded to be the IC_{50} value of EENH with 56.0±1.0% as the highest inhibition at 100 µg/mL, whereas IC_{50} of control was determined to be 62.70 µg/mL. These results indicate that EENH have good anti-inflammatory potential within the considered range when compared to aspirin as control.

CONCLUSION

Medicinal plants form the basis of traditional system of medicine, and are a research thrust area to be investigated for discovery of novel phytochemicals of biological importance. *N. herpeticum* is a least explored plant that has been known to use by various ethnic groups as a part of their delicacy or traditional remedy against ailments. The plant exhibited the presence of secondary metabolites like alkaloids, glycosides, phenolic compounds, terpenoids, and steroids. Here, we reported the acute oral toxicity profile of the plant, which was unascertain despite being used as edible plant. This study demonstrated that the ethanolic extract of *N. herpeticum* exhibited a concentration dependent inhibition of free radicals. The EENH showed potential antioxidant activity at higher concentrations. Also, the extract showed good anti-inflammatory activity *in vitro*. However, future *in vivo* study and computational tools are required to further investigate the pharmacological potential of the plant.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ABTS: 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); **BLAST:** Basic Local Alignment Search Tool; **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl; **EENH:** Ethanolic Extract of *Natsiatum herpeticum*; **GLP:** Good Laboratory Practice; **matK:** Maturase K; **OECD:** Organization for Economic Cooperation and Development; **rbcL:** Ribulose-1, 5-bisphosphate carboxylase/oxygenase large sub-unit.

REFERENCES

1. Chopra B, Dhingra AK. Natural products: A lead for drug discovery and development. Phytother Res. 2021;35(9):4660-702. doi: 10.1002/ptr.7099, PMID 33847440.
2. Kunakom S, Eustáquio AS. Natural products and synthetic biology: where we are and where we need to go. mSystems. 2019;4(3):e00113-19. doi: 10.1128/mSystems.00113-19, PMID 31120026.
3. Dzobo K. The role of natural products as sources of therapeutic agents for innovative drug discovery. Compr Pharmacol. 2022:408-22.
4. Atanasov AG, Zotchev SB, Dirsch VM, International Natural Product Sciences Taskforce, Supuran CT. Natural products in drug discovery: advances and opportunities. Nat Rev Drug Discov. 2021;20(3):200-16. doi: 10.1038/s41573-020-00114-z, PMID 33510482.
5. Pegu R, Gogoi J, Tamuli AK, *et al*. Ethnobotanical study of wild edible plants in Poba Reserved Forest, Assam, India: multiple functions and implications for conservation. Res J Agric For Sci. 2013;1:1-10.
6. Miya M, Adhikari A, Chhetri A. Wild edible plants consumed by different ethnic groups of Nepal-A review. Res J Agric For Sci. 2021;9:52-64.
7. Gogoi B, Zaman K. Phytochemical constituents of some medicinal plant species used in recipe during 'Bohag Bihu' in Assam. J Pharmacogn Phytochem. 2013;2:30-40.
8. Sonowal R. Some Folklore Medicines of the Sonowal Kachari tribe of Upper Assam, North East India. Scholars Acad J Biosci. 2014;2:541-3.
9. Nath KK, Deka P. Traditional remedies of Joint diseases in Assam. Indian J Trad Knowl. 2011;10:568-71.
10. Bose D, Roy JG, Mahapatra SD, *et al*. Medicinal plants used by tribals in Jalpaiguri district, West Bengal, India. J Med Plants Stud. 2015;3:15-21.
11. Basumatary S, Narzary H. Nutritional value, phytochemicals and antioxidant property of six wild edible plants consumed by the Bodos of North-East India. Mediterr J Nutr Metab. 2017;10(3):259-71. doi: 10.3233/MNM-17168.
12. Ramesha BT, Suma HK, Senthilkumar U, Priti V, Ravikanth G, Vasudeva R, *et al*. New plant sources of the anti-cancer alkaloid, camptothecine from the Icacinaceae taxa, India. Phytomedicine. 2013;20(6):521-7. doi: 10.1016/j.phymed.2012.12.003, PMID 23474217.
13. OECD. Test No. 423: acute Oral toxicity – Acute Toxic Class Method; 2002. Available from: https://www.oecd-ilibrary.org/environment/test-no-423-acute-oral-toxicity-acute-toxic-class-method_9789264071001-en, [accessed Jan 1 2023].
14. Gashaye MB, Birhan YS. Phytochemical constituents, antioxidant and antibacterial activities of *Plectocephalus varians* (A. Rich.) C. Jeffrey ex Cufod root extracts. BMC Complement Med Ther. 2023;23(1):135. doi: 10.1186/s12906-023-03919-8, PMID 37118732.
15. More GK, Makola RT. *In vitro* analysis of free radical scavenging activities and suppression of LPS-induced ROS production in macrophage cells by *Solanum sisymbriifolium* extracts. Sci Rep. 2020;10(1):6493. doi: 10.1038/s41598-020-63491-w, PMID 32300192.
16. Gunathilake KDPP, Ranaweera KKDS, Rupasinghe HPV. Influence of boiling, steaming and frying of selected leafy vegetables on the *in vitro* anti-inflammation associated biological activities. Plants (Basel). 2018;7(1):22. doi: 10.3390/plants7010022, PMID 29547518.
17. Gunathilake KDPP, Ranaweera KKDS, Rupasinghe HPV. *In vitro* anti-inflammatory properties of selected green leafy vegetables. Biomedicines. 2018;6(4):107. doi: 10.3390/biomedicines6040107, PMID 30463216.
18. Suriyatem R, Auras RA, Intipunya P, *et al*. Predictive mathematical modeling for EC50 calculation of antioxidant activity and antibacterial ability of Thai bee products. J Appl Pharm Sci. 2017;7:122-33.

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