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An investigation of traditional uses and anti-inflammatory property of *Clematis buchananiana* De Candolle and *Tupistra nutans* Wall. ex Lindl.: Native ethnomedicinal plants from Sikkim, India

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In the traditional medicinal system of Sikkim *Clematis buchananiana* and *Tupistra nutans* is used extensively to treat various ailments, however, they have not been validated for their anti-inflammatory property by *in vitro* method. Therefore, the present study was carried out to investigate specific ethnomedicinal uses, *in-vitro* anti-inflammatory property, and phytochemical constituents of *Clematis buchananiana* and *Tupistra nutans*. The ethnomedicinal usage was studied by calculating the value for fidelity level, use-value, and informant consensus factor. Stabilization of human red blood cell membrane and protein denaturation method was used to study anti-inflammatory property. The phytochemicals were analysed by the methods described elsewhere. *Clematis buchananiana* was found to be used more frequently for sinusitis, headache, cold and *Tupistra nutans* for high blood pressure, diabetes and stomach-ache. Both *C. buchananiana* and *T. nutans* was found to inhibit the HRBC membrane and protein denaturation effectively in a dose-dependent manner. However, inhibition of haemolysis and protein denaturation by *C. buchananiana* was found to be higher than *T. nutans* at all doses. The phytochemical screening revealed the presence of anti-inflammatory metabolites such as flavonoids and phenolics in both the plants. The results provide evidence for the anti-inflammatory property of *C. buchananiana* and *T. nutans.*

Keywords: *Clematis buchananiana*, Ethnomedicine, Inflammation, Sikkim, *Tupistra nutans*

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Inflammation is a common protective immunological reaction in response to tissue injury which is characterised by the accumulation of polymorphonuclear leukocytes and macrophages at the sites of tissue injury[1]. These cells contain large numbers of lysosomal granules in their cytoplasm which on release during inflammatory reactions can destroy pathogens as well as the normal cells and tissues leading to pathological condition[2]. Thus, stabilization of lysosomal membrane holds an important prospect to inhibit an inflammatory response[3]. Furthermore, denaturation of protein has been well documented in inflammation and arthritic diseases which results in the production of auto-antigens[4,5]. Although nonsteroidal anti-inflammatory drugs (NSAIDs) are often used to treat inflammation they come with various side effects[6,7].

In traditional medicine practices of Sikkim, about 420 plants are used to treat various ailments[8]. In the previous studies, elaborative documentation of medicinal use of plants from Sikkim has been made, out of which some are reported to be used to treat inflammatory diseases[9]. Moreover, only a handful of plants from Sikkim have been investigated for documentation of its anti-inflammatory property by experimental evidence-based method[10-13]. Among many of the plants used to treat various inflammation-related diseases, *Clematis buchananiana* belonging to the family Ranunculaceae and *Tupistra nutans* of the family Asparagaceae is being used in the traditional medicinal system of Sikkim. The genus *Clematis* was reported to be used for rheumatoid arthritis, bone disorder, chronic skin disease, muscle aches, colds, headaches, respiratory ailments etc.[14,15]. In the previous studies, the species of *Clematis* such as *Clematis erecta*[16], *Clematis chinensis*[17], *Clematis simensis*[18], *Clematis pickeringii*[19], *Clematis vitalba*[20] and *Clematis flammua*[21] have been investigated for anti-inflammatory property. Moreover, the anti-inflammatory property of *Clematis buchananiana* has not been performed to date. On the other hand, the genus *Tupistra* has been reported to be used for diabetes[22,37]. Moreover, only a few studies have been

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carried out so far to investigate the anti-inflammatory property of *Tupistra*[23].

Despite the use of *Clematis buchananiana* and *Tupistra nutans* in traditional medicinal practices of Sikkim the available literature shows that the species *C. buchananiana* and *T. nutans* have not been explored for its anti-inflammatory property by the *in-vitro* method. Therefore, the present study was carried out with the objectives to; (i) document the knowledge and ethnomedicinal usage of *Clematis buchananiana* and *Tupistra nutans* among the Sikkimese people, (ii) investigate the *in-vitro* anti-inflammatory property of methanolic extracts of *Clematis buchananiana* and *Tupistra nutans* by human red blood cell (HRBC) membrane stabilization and inhibition of protein denaturation method, (iii) screen the phytochemical constituent of *C. buchananiana* and *T. nutans*. The study may validate the traditional knowledge of the use of the plants as an anti-inflammatory agent.

Methodology

Documentation of ethno-medicinal usage

The documentation of traditional uses of *Clematis buchananiana* and *Tupistra nutans* was performed by administration of a standard questionnaire to 5 individuals from Namchi, South Sikkim; Ranka, East Sikkim; Shribadam, West Sikkim during the months of September to December 2018. The regions were selected on the basis of the availability of the plant *Clematis buchananiana* and *Tupistra nutans*. From the collected data informant consensus factor (*F*ic) was calculated using the formula by Heinrich *et al*. (1998)[24]. The use-value (UV) of each plant was calculated according to the formula by Phillips *et al*. (1994)[25]. Fidelity Level was calculated as per the formula provided by Friedmen *et al*. (1986)[26]. *Clematis buchananiana* (accession number-586) and *Tupistra nutans* (accession number-611) were collected from the above-mentioned areas of the Sikkim and brought to the laboratory of Department of Zoology, Sikkim University and was identified by a taxonomist from Department of Botany, Sikkim University.

Materials and methods

Plant extract preparation

The flowers of *T. nutans* and root of *Clematis buchananiana* were washed with distilled water to remove the dust particles and was initially dried at room temperature, avoiding direct sunlight and it was followed by drying in an incubator at 37°C. The dried plant material was ground into a fine powder. The extract of the plants was prepared in Soxhlet apparatus by mixing 10 gm plant powder with 400 ml methanol. The extract was then evaporated in a rotary evaporator at 42ºC followed by complete drying in an incubator at 37°C. The obtained plant extract was then stored at -20°C refrigerator until use.

Preparation of stock solution

The stock solutions of methanolic extract of *Clematis buchananiana* and *Tupistra nutans* was prepared as described elsewhere (Singh *et al*., 2019)[27].

Test for in-vitro anti-inflammatory property

All the assays were performed in triplicate for each of the different tests by the same investigator.

HRBC membrane protection test

About 5 ml fresh blood sample was collected from the investigator who was not under any dose of Non-Steroidal Anti-Inflammatory Drug (NSAID) for at least 15 days prior to the experiment. To the collected blood sample, an equal volume of Alsever's solution was added and centrifuged at 3000 rpm for 10 minutes after which supernatant was removed and a packed RBC pellet was obtained. It was washed three times with normal saline. The dissolved RBC pellets were reconstituted as 10% (v/v) suspension with iso-saline (pH=7.2). The reconstituted RBC was used as the stock erythrocyte (HRBC) suspension.

The standard protocol of Shinde et al. (1999)[28] with minor modifications was applied for heat-induced haemolysis test. For the assay methanolic extract of both the plants and standard drug (diclofenac) were prepared into the concentrations of 1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml and 5 mg/ml in distilled water. A reaction mixture was prepared to consist of 2 ml of Hyposaline (0.25% NaCl), 1ml of PBS (0.15 M, pH 7.4) and 1 ml plant extract or standard drug, 0.5 ml of 10% HRBC suspension. The control consisted of 1 ml distilled water instead of plant extracts, the other constituents were similar. The mixture was then incubated in an incubator for 30 min at 37°C and centrifuge at 2500 rpm for 5 mins. The supernatant was isolated gently and the absorbance was read at 560 nm in a UV-VIS spectrophotometer (Systronics). The HRBC membrane protection was calculated by the formula: -

Percentage of Protection= $100 - [(OD_1/OD_2) \times 100]$

Where, OD_1= Optical density of test sample

OD_2= Optical density of the control sample

Protein denaturation test

The *in-vitro* protein denaturation test was performed following the protocol of Mizushima *et al*. (1968)[29] and Sakat *et al*. (2010)[30] with slight modifications. The reaction mixture of 5 ml consisted of 0.2 ml of egg albumin, 2.8 ml of phosphate buffer saline (pH: 6.4) and 2 ml of varying concentrations of the plant extract or standard drug. For the controls, double distilled water was used instead of plant extracts. The reaction mixture was incubated at 37ºC in an incubator for 15 minutes followed by heating at 70ºC in a water bath. After cooling the absorbance was measured at 660 nm in UV-VIS spectrophotometer (Systronics). The percentage of inhibition of protein denaturation was calculated by the formula: -

Percentage of inhibition = [1- (Abs_{sample}/ $Abs_{control}$)] x 100

Where, Abs_{sample} = Absorbance of the Sample

$Abs_{control}$ = Absorbance of the Control

Phytochemical Analysis

The qualitative phytochemical analysis was performed with powdered plant material and methanolic extract of the plants as per the protocol described elsewhere (Parekh and Chanda, 2007)[31]. The quantitative tests for flavonoids and phenolic phytoconstituents were performed as described by Abbasi *et al*. (2015)[32].

Statistical analysis

The data analysis was performed using IBM SPSS 23. Shapiro-Wilk test was first applied to examine the normality of the data. The variables were expressed as mean and Standard Error Mean (S.E.M). One-way analysis of variance was used to determine a statistically significant difference between plant extracts and standard drug in membrane protection and protein denaturation. Pair-wise Karl Pearson correlation test was performed to analyze the relations between the effects of methanolic extracts and diclofenac on membrane protection and protein denaturation. The p-value of <0.05 was considered statistically significant.

Results

Documentation of ethno-medicinal knowledge and usage

The results show that *Clematis buchananiana* and *Tupistra nutans* are used to treat various ailments by the people of Sikkim and they have an important place in the traditional medicinal system. It was noted that for *Clematis buchananiana* the plant parts used by people for the medicinal purpose was the root and for *Tupistra nutans* flowers and fruits. However, among the two species, *Clematis buchananiana* (UV= 0.4) was found to be used most commonly for the medicinal purposes to treat sinusitis, headache, cold, etc. (Table 1). On the other hand, *Tupistra nutans* (UV=0.3) was reported to be used for the treatment of diabetes, high pressure etc. The fidelity level (FL) of *Clematis buchananiana* was found higher in sinusitis (68%), followed by headache/migraine (36%). However, for *Tupistra nutans* fidelity level was high for high blood pressure (64%) followed by diabetes (60%). Even though the informant consensus **(**Fic) value for most of the ailment mentioned was one (1) however, for headache and gastric problems it was 0.9 and 0.5 respectively (Table 1).

In-vitro anti-inflammatory assay

Hypotonicity induced HRBC membrane stabilization test

The results of the HRBC membrane stabilization test is presented in Table 2. The results show that both the methanolic extract of *Clematis buchananiana* and *Tupistra nutans* has the capacity to inhibit the haemolysis of HRBC. However, *C. buchananiana* was found to have a higher percentage of inhibition to HRBC haemolysis than *T. nutans* at all the concentrations. Moreover, the inhibition of haemolysis was found to be higher for the standard drug than the plant extracts at all the concentrations. At the highest concentration (5 mg/ml), the inhibition of haemolysis induced by diclofenac (standard drug) and *C. buchananiana* exhibited almost similar value. One-way ANOVA analysis revealed that there was no significant difference in protection between *C. buchananiana* and standard diclofenac drug (F= 4.227, df=2, p=0.471). However, the protection achieved by *T. nutans* was significantly lower than the standard drug (F= 4.227, df=2, p=0.033). Shapiro-Wilk test shows the sample data are normally distributed (0.805). Karl-Pearson correlation test for inhibition of haemolysis between plant species and drug in varying concentration was found to be dose-dependent (Table 3). However, *C. buchananiana* (r=1, p=0.001) showed strong correlation with dose than *Tupistra nutans* (r=0.994, p=0.001).

Protein denaturation test

Results of the protein denaturation test are presented in Table 2. Shapiro-Wilk test revealed that the sample data are normally distributed (0.603). The methanolic extracts of *Clematis buchananiana* and

Table 1 — Fidelity level (FL), use-value (UV) and informant consensus factor (Fic) values of *Clematis buchananiana* and *Tupistra nutans* for various ailments.

Plants	Total respondents	Illness Treated	FL (%)	Use value (UV)	Illness Treated by either *C. buchananiana* or *T. nutans*	Number of use-reports (Nur)	Number of Taxa Used (Nt)	Fic
Clematis buchananiana (Pinaase lahara)	25	Cold	32	0.4	Cold	8	1	1
		Nose block	12		Nose block	3	1	1
		Sinusitis	68		Sinusitis	17	1	1
		Nose bleeding	24		Nose bleeding	6	1	1
		Headache/ migraine	36		Headache/ migraine	10	2	0.9
		Gastric	4		Gastric	3	2	0.5
		Toothache	20		Toothache	5	1	1
		Jaundice	8		Jaundice	2	1	1
		Tissue pain	8		Tissue pain	2	1	1
		Diabetes	60		Diabetes	15	1	1
Tupistra nutans (Nakima)	25	Stomachache	16	0.3	Stomachache	4	1	1
		High blood pressure	64		High blood pressure	16	1	1
		Food Poison	4		Food Poison	1	1	1
		Pneumonia	4		Pneumonia	1	1	1
		Fever	12		Fever	3	1	1
		Headache	4					
		Gastric	8					

Table 2 — Effect of methanolic extracts of *Clematis buchananiana*, *Tupistra nutans* and diclofenac in the protection of HRBC membrane and egg albumin protein denaturation [n=3 in each concentration].

Concentrations	% of HRBC membrane inhibition			% of Inhibition of Protein Denaturation		
	Clematis buchananiana (Mean±SEM)	*Tupistra nutans* (Mean±SEM)	Diclofenac (Mean±SEM)	*Clematis buchananiana* (Mean±S.E.M)	*Tupistra nutans* (Mean±S.E.M)	*Diclofenac drug* (Mean±S.E.M)
1mg/ml	59.2 ± 1.6	53.7±9.7	68.4 ± 2.7	33.1 ± 3.5	18.5 ± 2.7	38.2 ± 5.7
2mg/ml	64.7 ± 0.2	57.7 ± 10.0	72.4 ± 3.3	40.5 ± 7.5	22.2 ± 0.8	70.5 ± 10.6
3mg/ml	70.7 ± 1.3	64.0 ± 11.6	77.9 ± 2.1	46.3 ± 8.8	26.3 ± 2.4	75.4 ± 12.0
4mg/ml	76.4 ± 0.7	67.1 ± 11.9	80.3 ± 3.2	51.1 ± 10.1	38.9 ± 7.8	83.1 ± 10.3
5mg/ml	82.5 ± 0.3	70.9 ± 9.9	83.2 ± 2.6	59.9 ± 6.8	53.3 ± 13.1	89.4 ± 5.0

Table 3 — Karl Pearson's correlation coefficient (r) test for dose-dependent pairwise correlation between drug, *Clematis buchananiana* and *Tupistra nutans* with the protection of HRBC membrane and protein denaturation.

Treatment	HRBC membrane inhibition		Inhibition of Protein Denaturation	
	r- value	p-value	r- value	p-value
C. buchananiana vs Concentration	1.000	0.001	1.000	0.001
T. nutans vs Concentration	0.994	0.001	1.000	0.001
Diclofenac vs Concentration	0.989	0.001	1.000	0.001

Tupistra nutans inhibited the denaturation of egg albumin effectively. However, the higher percentage of inhibition of protein denaturation was observed for *Clematis buchananiana* than *Tupistra nutans*. However, the inhibition of egg albumin denaturation by diclofenac drug (89.4%) was higher than the plant extracts. One-way ANOVA analysis suggested that there was no significant difference between the inhibitions of protein denaturation by *C. buchananiana* and diclofenac drug (F= 8.535, df=2, p=0.056). However, there was a significantly higher inhibition of protein denaturation by standard diclofenac drug than the *methanolic extract of T. nutans* (F= 8.535, df=2, p=0.004).

Phytochemical Screening

The results of the phytochemical analysis are presented in Table 4. The quantitative phytochemical

Table 4 — Phytochemical screening and quantitative test of flavonoid and phenolic content from the roots of *Clematis buchananiana* and flowers of *Tupistra nutans.*

Phytochemicals	**Results**	
	C. buchananiana	***T. nutans***
Flavonoids	+	+
Tannins	+	+
Phenolics	+	+
Terpenoids	+	+
Steroids	-	+
Glycosides	-	+
Anthraquinones	-	+
Coumarines	+	+
Anthocyanins	+	+
Total Flavonoid Content (mgRt/100g, FW)	63.89	47.87
Total Phenolic Content mgGAE/100g, FW)	17.17	18.59

*The sign '−' means absent and '+' means present

screening of dried powder and methanolic extracts from the roots of *C. buchananiana* and flower of *T. nutans* showed the presence or absence of certain phytoconstituents. The quantitative analysis of total flavonoid content (TFC) in the root of *Clematis buchananiana* and flower of *Tupistra nutans* was found to be 63.89 mgRt/g, Fw and 47.87 mgRt/g, Fw respectively. Further, the total phenolic content in *C. buchananiana* and *T. nutans* was found to be 17.17 mg GAE/g, FW and 18.59 mg GAE/g, FW respectively.

Discussion

Medicinal plants have long been recognized as an important source of therapeutically beneficial compounds. In the earlier study, Pradhan *et al*. (2008) reported the use of roots of *Clematis buchananiana* for treating sinusitis and headache and the inflorescence of *Tupistra nutans* to treat the body pain[33]. In the present study, similar medicinal uses of *Clematis buchananiana* and *Tupistra nutans* as herbal medicine have been documented. Further, the use of *C. buchananiana* roots and inflorescence of *T. nutans* was also reported by Idrisi *et al*. (2010) in their study[9]. However, they have not analysed the Fidelity level, consensus factor (Fic) and use value (UV) in their study. The present study suggested that the use of species *Clematis buchananiana* is higher (UV=0.4) than *Tupistra nutans* (UV=0.3) among the people of Sikkim. Furthermore, *Clematis buchananiana* was found to be used more frequently to treat sinusitis followed by headache and cold as suggested by high FL value. However, unlike the widely available literature for use of *Tupistra nutans* for diabetes, the present study observed a higher FL value (64%) for high blood pressure than diabetes, showing its maximum use to treat high blood pressure which can be considered as a new finding.

The lysosomal enzymes released during inflammation cause a variety of disorders that are related to acute or chronic inflammation. Thus, stabilization of lysosomal membrane is an important step to stop inflammatory response by inhibiting the release of lysosomal constituents (bactericidal enzymes and proteases) of activated neutrophil which promotes tissue inflammation and damage upon extracellular release[8]. As the membrane of HRBC resembles the membrane of lysosome the HRBC membrane stabilization method was used in the present investigation to evaluate the anti-inflammatory property of *C. buchananiana* and *T. nutans*. Further, the denaturation of protein is well documented in inflammation and the protection of denaturation of proteins by NSAIDs was reported by Mizushima (1964)[34]. As such, the inhibition of protein denaturation by the plant extract was evaluated by various researchers to investigate the anti-inflammatory activity[5,35]. Therefore, in the present study, HRBC membrane stabilization and inhibition of albumin protein denaturation method were followed to evaluate the anti-inflammatory property of *C. buchananiana* and *T. nutans*.

The results of the present study provide evidence for the anti-inflammatory property of *Clematis buchananiana* and *Tupistra nutans*. However, *C. buchananiana* was found to have a higher potential to stabilize the HRBC membrane (Fig. 1) which suggests a higher potency of *C. buchananiana* as an anti-inflammatory agent. Moreover, *C. buchananiana* and *T nutans* could effectively inhibit the protein denaturation induced by heat which suggests that it can prevent the loss of biological function of

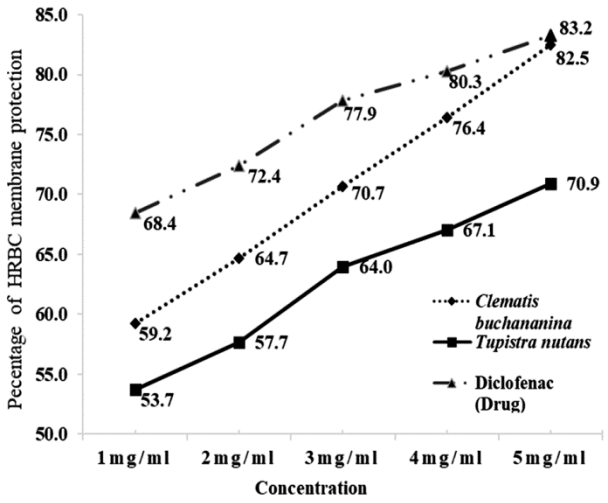

Fig. 1 — Graphical representation of the percentage of HRBC membrane protection by methanolic extracts of *Clematis buchananiana*, *Tupistra nutans* and diclofenac at different concentrations.

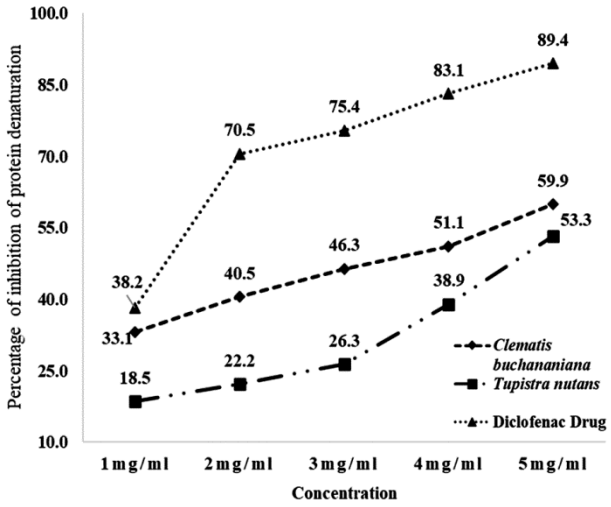

Fig. 2 — Graphical representation of inhibition of protein denaturation by methanolic plant extracts of *Clematis buchananiana*, *Tupistra nutans* and diclofenac at different concentrations.

proteins[35] (Fig. 2). Various other species from the genus *Clematis* have been reported to possess anti-inflammatory properties[17,18,21,36]. However, previous studies were performed by *in-vivo* methods such as tail immersion test and carrageenan-induced paw oedema model in mice[36], whereas in the present investigation *C. buchananiana* was studied for the anti-inflammatory property by *in-vitro* HRBC membrane stabilization and protein denaturation method. In the previous studies, it was reported that *Clematis chinensis* has the potential to inhibit the inflammation by inhibiting the expression of nuclear factor kappa B (NF-κB) p65 subunits, TNF-α and COX-2[17]. According to pharmacophylogeny, the plants with close genetic relatedness can have similar chemical components[15]. As *C. chinensis* and *C. buchananiana* belong to the same genus, it can be supposed that the anti-inflammatory effect of *C. buchananiana* may exhibit a similar mechanism of inhibitory effect as *C. chinensis*. Studies have revealed that the non-polar hexane fraction of *Clematis flammua* can inhibit the cytokine biosynthesis[21]. Furthermore, the anti-inflammatory property of the aerial parts of *Clematis vitalba* was found to be due to the presence of 4-O-coumaroyl-isovitexine (Vitalboside)[20]. Thus, the presence of certain specific compound is well known for having anti-inflammatory activity in *Clematis sp.* Even though the presence of these specific molecules was not investigated in the present study the anti-inflammatory activity of *C. buchananiana* can be speculated to be due to the presence of such specific compounds which can inhibit the pro-inflammatory cytokines.

The available literature shows that the study of the anti-inflammatory effect of *Tupistra* by HRBC membrane stabilization method is very less. In the present investigation even though *Tupistra nutans* was found to have an anti-inflammatory effect but it was lesser than *C. buchananiana* which may be due to its lesser amount of total flavonoid content. Moreover, the family Asparagaceae to which *Tupistra* belongs has species having an anti-inflammatory property[15]. In addition to its anti-inflammatory property *Tupistra nutans* is also known to have antidiabetic[37] and antioxidant property[38] due to which it is often used as an anti-diabetic agent and it is also evident from the higher FL value in the present study (Table 1). Studies have reported the presence of compound-21 in the rhizome of *Tupistra chinensis* which was found to inhibit NO production during inflammation[39]. The study of *Agave sisalana* belonging to the family Asparagaceae revealed the presence of the steroidal molecules hecogenin and tigogenin which was found to inhibit inflammation by reducing the levels of histamine, serotonin and other inflammatory mediators and maintain the prostaglandin E_2 (PGE_2) and leukocyte filtration[40]. In the present investigation, *T. nutans* was found to contain steroids and flavonoids (Table 4) which are known as anti-inflammatory phytochemicals.

The preliminary phytochemical screening of the plant extracts revealed the presence of flavonoids, triterpenes, tannins, phenolics, coumarins in methanolic

extracts of both *C. buchananiana* and *T. nutans*. Moreover, the presence of steroids, anthraquinones, glycosides were confirmed in *T. nutans*. The results were in accordance with the previous study in *T. nutans*[41]. However, the phytochemical screening of *C. buchananiana* revealed the presence of phenolic acids which was not reported in the previous study by Subba *et al*. (2018)[42]. The presence or absence of phytochemicals may differ due to the differences in geographical location, age difference in individual plants, differences in topographical factors and the nutrient concentrations of the soil which may be the reason behind variation in phytoconstituents and their concentration[43]. This might be the reason for non-agreement of the present study with that of Subba *et al*. (2018), as the species studied by Subba et al. (2018) was from Nepal and in the present study, *C. buchananiana* was collected from Sikkim, India[42]. Moreover, the presence of phenolic acids and other phytochemical compounds from the genus *Clematis* is in agreement with the previous investigation[44,15]. The flavonoids and phenolic acids are the phytoconstituents of the polyphenolic family which act against inflammation[45] by inhibiting the production of pro-inflammatory molecules such as TNF-α, leukocyte adhesion factor and nitric oxide (NO) produced during inflammation[46]. The anti-inflammatory activity also depends upon the levels of total flavonoids and phenolic significantly[46]. In the present study, the maximum total flavonoid content was found in *C. buchananiana*, whereas the maximum total phenolic content was found in *Tupistra nutans*. Thus, the higher level of anti-inflammatory activity shown by *C. buchananiana* can be correlated with a higher level of total flavonoid content. Moreover, the anti-inflammatory activity of *C. buchananiana* and *T. nutans* are probably due to the presence of flavonoids, coumarins, phenolics, and terpenoids as these phytochemicals are found to possess effective anti-inflammatory and antioxidant properties. In addition, the anti-inflammatory activities of *C. buchananiana* and *T. nutans* are probably due to the inhibitory effects by phytochemicals on enzymes involved in the production of chemical mediators of inflammation and metabolism of arachidonic acid[47].

The present study was undertaken *in-vitro* condition and *in-vivo* model was not used to assess the anti-inflammatory activity of the plant extracts. Moreover, only a few qualitative phytochemical screenings were performed from the plant extract and the phytochemical screening for saponins was not performed that was known to be responsible for anti-inflammatory effect in some species of genus *Clematis* and *Tupistra*[39,48]. The study of specific compounds and their effects that are responsible for the anti-inflammatory activity by *C. buchananiana* and *T. nutans* was not performed in the present study. Considering these limitations present study provides the evidence for the anti-inflammatory property possessed by *C. buchananiana* and *T. nutans*.

Conclusions

The results of the present study provide an evidence-based boost for traditional knowledge of the usage of *C. buchananiana* and *T. nutans* in inflammation related diseases, for which it is used widely by the people of Sikkim. Future studies are warranted with more sophisticated techniques to understand the mechanism action of these plants against inflammation.

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Conflict of Interest

Authors declare there is no conflict of interest

Authors' Contribution Statement

BS conceptualized the study design and have written the manuscript, MB carried out the laboratory experiment, and JG carried out the statistical analysis.

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