

REVIEW

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Tupistra Nutans: A comprehensive review of morphology, phytochemical profile, and Pharmacological activity"

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Abstract

Background *Tupistra nutans*, commonly known as Nakima in the Sikkim region of India, is a fascinating plant species with potential medicinal and culinary uses. It belongs to the family Asparagaceae.

Main body It's native to the Eastern Himalayas and is prized for its distinctive appearance and nutritional value. Recent research has identified *Tupistra nutans* is having potential phyto constituents such as Unsaturated fatty acids, Vitamin E, Diosgenin, Linoleic acid and Palmitoleic acid. *Tupistra nutans* is a unique plant with a distinctive appearance. It is a perennial herb with a rhizomatous root system. The Leaves are large, strap-shaped and can reach up to 1–2 m in length. They are dark green, glossy and arranged in a basal rosette. The Inflorescence is a unique, club-shaped inflorescence which bears numerous small, tubular flowers. The Colour can vary but is often a striking yellow or orange. The Fruits are berry-like fruits that are typically red or orange when ripe. Traditionally *Tupistra nutans* has Culinary Use like the inflorescence is a popular vegetable, often cooked in curries or stir-fries. It is prized for its unique texture and flavour, often compared to button mushrooms. The Medicinal Use in pain relief, blood sugar regulation, anti-inflammatory properties, antioxidant properties and anti-diabetic properties.

Conclusion While scientific research is still in its early stages, the plant's antioxidant, anti-inflammatory, and antidiabetic properties warrant further exploration. By understanding and harnessing the power of this indigenous plant, we can unlock its potential for sustainable development, healthcare, and culinary innovation.

Background

Tupistra nutans, a captivating evergreen herb belonging to the Asparagaceae family, has a history shrouded in botanical mystery. Initially documented in 1829 from the Sylhet region of Bangladesh, its true distribution remained elusive for nearly two centuries [1]. Early accounts from India's Khasia Hills in 1871 suggested its presence, but subsequent reports of sightings in Sikkim (1975) and Bhutan (1994) proved to be erroneous [2]. Ultimately, a fortuitous rediscovery in 2006 within the Nokrek Biosphere Reserve, nestled in Meghalaya, clarified its true range. This revelation confirmed its exclusive

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habitat– the lush forest floors of the Khasia Hills and Sylhet [2].

The genus *Tupistra*, comprising 31 diverse species, primarily thrives in the southern and southeastern regions of Asia, spanning countries such as Nepal, Bhutan, India, Myanmar, China, Laos, Vietnam, Thailand, and Malaysia. Within India, nine species have been identified: *Tupistra ashihoi*, *Tupistra Clarkei*, *Tupistra khasiana*, *Tupistra leonidii*, *Tupistra nutans*, *Tupistra nagarum*, *Tupistra extrorsandra*, *Tupistra stoliczka*, and *Tupistra tupistroides*. These species are distributed across various Indian states, including Meghalaya, Assam, Nagaland, Sikkim, and Tripura. Notably, six of these species are endemic to Northeast India, highlighting the region's remarkable biodiversity [3].

However, the true value of *Tupistra nutans* lies in its traditional medicinal use. Revered as “Nakima” in Himalayan communities, it has been employed as a natural remedy for generations, particularly for managing blood sugar [4, 5] and pain [5]. Recent scientific research has begun to unravel the herb's secrets, revealing a treasure trove of bioactive compounds within its leaves. These compounds, including saponins, phenolic acids, and flavonoids, contribute to its potent antioxidant, anti-inflammatory, and antimicrobial properties, promising significant potential for modern medicine [4, 5].

Beyond its medicinal prowess, *Tupistra nutans* offers a fascinating cultural connection. Its succulent flower buds are a seasonal delicacy, gracing curries and pickles with their unique flavour, demonstrating the deep-rooted relationship between Himalayan communities and their natural resources [6].

Despite its historical and potential significance, indigenous knowledge surrounding *Tupistra nutans* is at risk of extinction. Relied upon oral tradition, this invaluable heritage is vulnerable. Documenting these practices is essential not only for historical preservation but also to unlock the plant's full potential in modern medicine and sustainable use. Recent studies confirming its antioxidant properties open new avenues for research and sustainable development [2].

Tupistra nutans emerges as a compelling story of rediscovery, traditional wisdom, and scientific potential. Its journey from obscurity to botanical intrigue highlights the importance of preserving both cultural heritage and natural resources. By delving deeper into the secrets of this Himalayan treasure, we can ensure its sustainable use and responsible development, perpetuating its legacy of healing for future generations [4].

Methodology

A total no of 900 records from the data bases and 15 records from other sources were screened initially. The 350 records were excluded because the quality of the

data was poor and less authentic. Another 250 and 171 records were included as they are irrelevant and not specific for the topic. The total no of 144 records were utilized to draft this manuscript.

Vernacular name [7].

Nepali: Nakima (aule).

Lepcha: Purfeg.

Detailed morphological description of *Tupistra Nutans*[1, 3, 8–10]

Plant type

- It is a terrestrial perennial herb.

Stem

- The plant has a rhizomatous stem which is cylindrical and ascending. The diameter of the stem is between 1.5 and 1.7 cm.

Cataphylls

- The cataphylls are linear-lanceolate, with a length of up to 24 cm and a breadth of 1.5 cm.

Leaves

- The plant produces one or two leaves annually from the shoot apex.
- The leaves are narrowly oblanceolate or narrowly oblong-oblanceolate, with a length of up to 112 cm and a width between 9.5 and 12 cm.
- The apex of the leaves is acute or acuminate, and they taper gradually to the petiole.

Peduncle

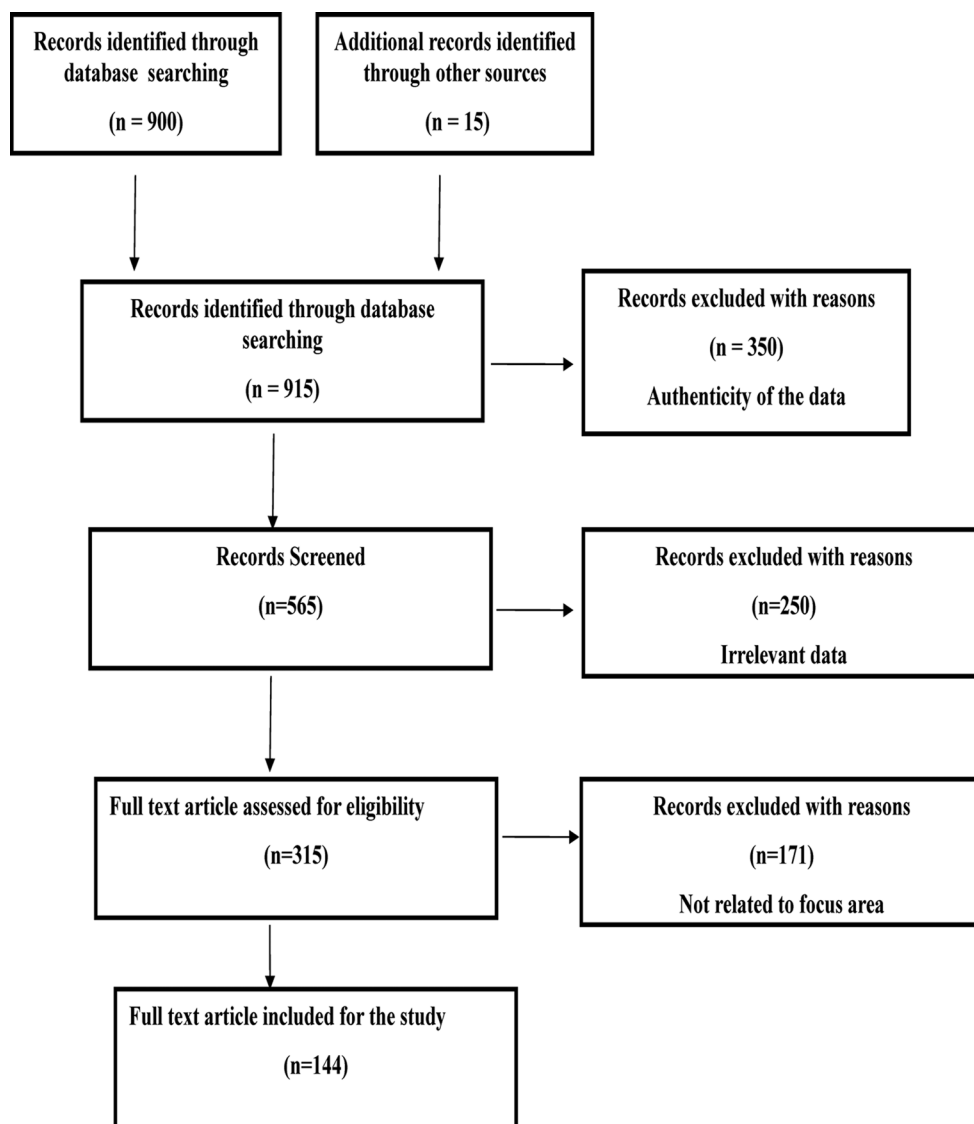
- The peduncle is green, erect, slightly declinate distally, and terete.
- It is usually 3–4 times longer than the spike, with a length between 17 and 25 cm.
- It arises from the apical part of the stem and is axillary.

Spike

- The spike is pendulous and densely flowered with up to 23 flowers. It has a length between 5 and 7 cm.

Bracts

- There are two bracts per flower. The outer bract is borne below and adjacent to the flower and is lanceolate, deltoid-ovate or ovate.

**Fig. a**

- It has a length between 7 and 12 mm and a breadth of up to 8 mm.
- The inner bract (bracteole) is lanceolate, with a length up to approximately 6 mm. It is borne laterally to the flower.

Flowers

- The flowers are sessile. The perianth is campanulate, 6-cleft, and fleshy. The tube is 5–6 mm long.
- The lobes are narrowly ovate, and recurved, with a length of 6–6.5 mm and breadth between 3 and 4.5 mm.
- They are pale yellowish brown to greenish with purple streaks/dots ventrally.

Stamens

- There are six stamens. The anthers are subsessile, dorsifixed, oblong, and introrse.
- They are inserted at the apex of the perianth tube and are concealed by the stigma.
- They have a length between 1 and 1.5 mm.

Pistil

- There is one pistil. The style is columnar, with a length between 4.5 and 6 mm.
- The stigma is white, sub-capitate, with 3-lobes and an undulated limb. It has a diameter between 3 and 6 mm.

Table 1 Taxonomy [1, 11]

Kingdom	Plantae
Phylum	Streptophyta
Class	Equisetopsida
Subclass	Magnoliidae
Order	Asparagales
Family	Asparagaceae
Genus	<i>Tupistra</i>
Species	<i>Tupistra nutans</i>

- The ovary is superior, situated at the base of the columnar pistil, and is approximately 1 mm high. It is 3-locular.

Flowering

- The plant flowers between September and October.

Distribution

- The plant is found in India (specifically in the Khasi and Garo Hills of Meghalaya) and Bangladesh (Sylhet).

Comparative analysis of *Tupistra nutans* with similar species

In botany, closely related species often share similar characteristics, making identification challenging. This is particularly true for species within the *Tupistra* genus. This section focuses on *Tupistra nutans*, highlighting its unique features to differentiate it from its close relatives: *T. clarkei*, *T. leonidii*, *T. nagarum*, *T. tupistroides*, *T. khasiana*, *T. fungilliformis*, and *T. pingbianensis* [1, 8]. This comparative analysis provides clear differentiation criteria to aid in the accurate identification of *T. nutans* (Table 2)(Fig. 1).

Table 2 Difference between *Tupistra nutans* and other species of genus *Tupistra* [1, 8, 9, 12]

Character-istic	<i>Tupistra nutans</i>	<i>Tupistra clarkei</i>	<i>Tupistra leonidii</i>	<i>Tupistra tupistroides</i>	<i>Tupistra nagarum</i>	<i>Tupistra khasiana</i>	<i>Tupistra fungilliformis</i>	<i>Tupistra pingbianensis</i>
Peduncle	Erect, typically 3–4 times longer than the spike	Pendulous, nearly as long as the spike	Com-paratively longer	Erect, usually 3–4 times longer than the spike	Erect to decli-nate distally, 17–25 cm long, 3–4 times spike length	Erect to decli-nate distally, 17–25 cm long, 3–4 times spike length	Erect to decli-nate distally, 17–25 cm long, 3–4 times spike length	Erect to decli-nate distally, 17–25 cm long, 3–4 times spike length
Spike	Densely flow-ered, up to 23 flowers within a 5–7 cm length	30-flowered, up to 11 cm long	Shorter, fewer flowers	Densely flow-ered, up to 23 flowers	5–7 cm long, up to 23 flow-ers per spike	5–7 cm long, up to 23 flowers per spike	5–7 cm long, up to 23 flow-ers per spike	5–7 cm long, up to 23 flow-ers per spike
Stigma	Sub-capitate and white, large enough to con-ceal the anthers	Peltate, apically nearly flat, dark purple, not large enough to conceal the anthers	Subcapi-tate and white, devoid of purple spots	White, sub capi-tate with 3 lobes and an undulate limb	Hemispheric, with den-ticulation at the margin, white	Hemispheric, with denticula-tion at the mar-gin, white	Hemispheric, with denticula-tion at the margin, white	Hemispheric, with denticu-lation at the margin, white
Perianth lobes	Narrowly ovate lobes, 6–6.5 mm, transition-ing from pale yellow-brown to green, with purple markings underneath	ovate to broadly ovate, 7.5–10 mm, pale fulvous to creamy with purple underside	-	Narrowly ovate, pale yellowish brown to green-ish with purple streaks/dots ventrally	Narrowly ovate and 6–6.5 mm long, exhibit a pale yellow-ish brown to greenish hue with purple ventral streaks or dots.	Narrowly ovate lobes, 6–6.5 mm, transition-ing from pale yellow-brown to green, with purple markings underneath	Narrowly ovate lobes, 6–6.5 mm, transitioning from pale yellow-brown to green, with purple markings underneath	Narrowly ovate lobes, 6–6.5 mm, transitioning from pale yellow-brown to green, with purple markings underneath
Flower	The flowers are antrorse, or di-rected forwards	The flowers are retrorse, or directed backwards	-	The flowers are antrorse, or di-rected forwards	The flowers are antrorse, or directed forwards	-	-	-
Anthers	Less distinctly stipitate	Distinctly stipitate	-	-	-	The large stigma hides it inside the perianth tube.	The large stigma hides it inside the perianth tube.	The large stigma hides it inside the perianth tube.



Fig. 1 Different Parts of *Tupistra nutans*

Phytochemical Profile

Fatty acid

“A GC-MS analysis of *Tupistra nutans* inflorescence extract revealed a remarkable fatty acid profile: two-thirds of the total content consisted of unsaturated fatty acids (UFAs), while the remaining third was saturated fatty acids (SFAs). This high UFA proportion suggests potential health benefits associated with its consumption [13].

Oleic acid, linoleic acid, and elaidic acid were the predominant UFAs identified. Oleic and linoleic acids are well-known for their cardioprotective properties, including reducing LDL and non-HDL cholesterol levels and exhibiting potent antioxidant activity. They achieve this by scavenging free radicals and mitigating pro-inflammatory signaling pathways without increasing lipoprotein levels [13–16] (Fig. 2).

However, elaidic acid, a trans fatty acid, has been linked to pro-inflammatory activity, which could potentially counteract the beneficial effects of other UFAs [17]. Therefore, the relative abundance of each UFA is critical in determining the overall impact on cardiovascular disease risk.”

Vitamin E

Vitamin E, a potent antioxidant and anticancer agent [18, 19], has been uniquely identified in the flower clusters of *Tupistra nutans*, a plant native to the Eastern Himalayas [13]. The methanolic extract of the inflorescence (METNI) exhibits antioxidant activity due to the

presence of Vitamin E and other natural antioxidants [13, 20]. Additionally, METNI has demonstrated anticancer potential against liver cancer cells, likely due to a synergistic effect between Vitamin E and other bioactive compounds [13].

Interestingly, Vitamin C was exclusively detected in the inflorescence of *T. nutans* [13]. Among the 28 compounds identified in METNI through GC-MS, 12% of the relative abundance was contributed by natural antioxidants like Vitamin E, diosgenin, and phenol [13]. METNI exhibited antioxidant activity in HepG2 and F111 cells due to the presence of natural antioxidants, including Vitamin E and some unsaturated fatty acids [13]. However, at higher concentrations, METNI induced oxidative stress [13]. This paradoxical behavior of Vitamin E has been reported in another study [21].

Given the high concentration of Vitamin E and its proven anticancer properties, further research is needed to elucidate the mechanisms underlying its cancer cell inhibition and to explore strategies for mitigating oxidative stress at higher concentrations. The current evidence suggests that Vitamin E in *T. nutans* holds significant potential for promoting human health and well-being.

Diosgenin

GC-MS analysis has revealed that the inflorescence of *Tupistra nutans* is a significant source of diosgenin (2.63%), a promising biomolecule with a diverse range of potential health benefits. While diosgenin is primarily

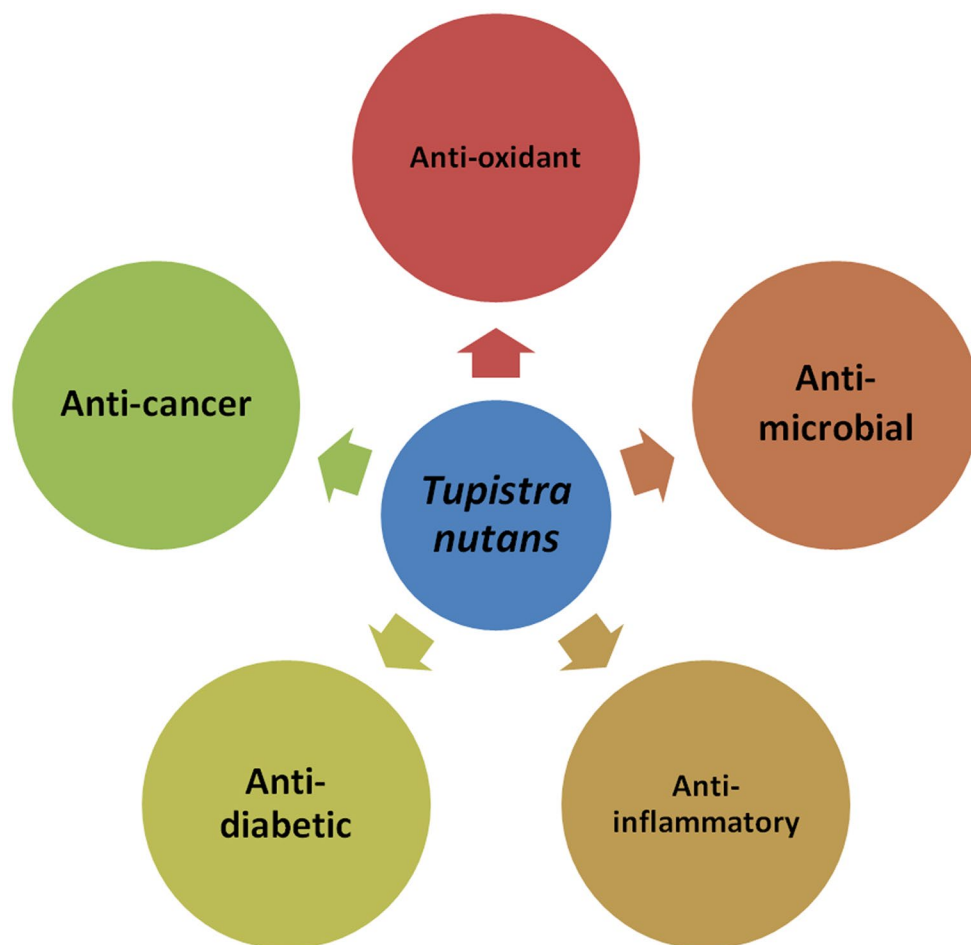


Fig. 2 Pharmacological Actions of *Tupistra nutans*

found in the root and inflorescence extracts, studies have shown its potential to:

- Inhibit cancer cell growth: Diosgenin targets key signaling pathways like NF- κ B and STAT3, disrupting cell cycle regulation.
- Protect cardiovascular health: It helps regulate blood sugar and cholesterol levels, reducing the risk of heart disease.
- Shield neurons: Diosgenin may protect neurons from damage, promoting cognitive function.
- Boost immunity: It can strengthen the immune system, enhancing the body's defense mechanisms.
- Promote skin health: Diosgenin may offer benefits for skin health, such as wound healing and anti-aging.
- Reduce inflammation and oxidative stress: It has potent anti-inflammatory and antioxidant properties.

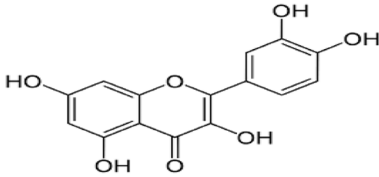
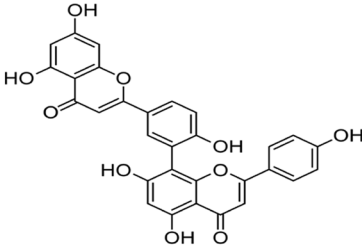
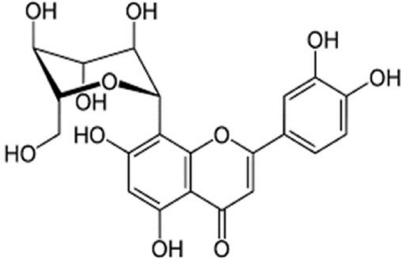
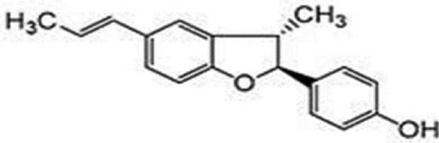
While in vitro and animal studies are promising, further clinical trials are necessary to fully understand the therapeutic potential of diosgenin in humans. By harnessing

the power of diosgenin from *T. nutans*, we can pave the way for improved individual and public health.

Linoleic acid

The methanolic extract obtained from *Tupistra nutans* inflorescence, analyzed using GC-MS, revealed a diverse array of compounds. Among these, linoleic acid constitutes more than 1% of the retention area [13]. Linoleic acid, classified as a polyunsaturated omega-6 fatty acid, is renowned for its health benefits [24]. Research indicates that it possesses anticancer, anti-inflammatory, and antioxidant properties [16, 25]. Moreover, linoleic finds application in photoprotective formulations, commonly used in various cosmetic products [26]. Its ability to modulate proinflammatory signalling further underscores its role as an antioxidant [27]. Despite inducing higher intracellular ROS generation at elevated extract concentrations, the extract exhibited greater inhibition of cell proliferation in cancerous cells compared to normal cells¹. This intriguing phenomenon highlights *Tupistra nutans* as a potential natural anticancer agent [13].

Table 3 Compound name and structure of flavonoids present in *Tupistra nutans*

Sl. no	Compound name	Structure
1	Quercetin	
2	Amentoflavone	
3	Orientin	
4	Conocarpan	

In summary, the diverse phytochemical composition of *Tupistra nutans*, particularly its linoleic acid content, points toward promising therapeutic applications in oncology, dermatology, and cosmetology. However, comprehensive studies are necessary to unravel the underlying mechanisms and validate its clinical efficacy.

Polyphenol

The investigation of plant extracts has revealed a rich source of bioactive compounds. Notably, root extracts of certain plants have been found to contain significant amounts of polyphenols, primarily salicylic acid and p-coumaric acid [28].

Table 4 Compound name and structure of polyphenols present in *Tupistra nutans*

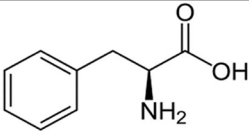
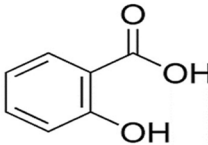
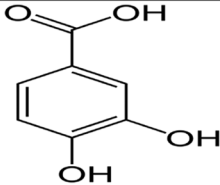
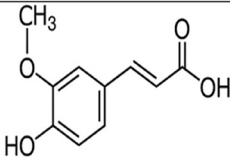
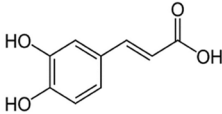
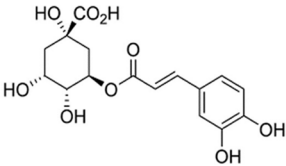
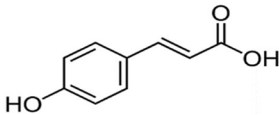
SL no	Compound name	Structure
1	1-phenylalanine	
2	Protocatechuic acid	
3	p-hydroxybenzoic acid	
4	Ferulic acid	
5	Caffeic acid	
6	Chlorogenic acid	
7	P-Coumaric acid	

Table 5 Concise summary of In-Vitro assay results for *Tupistra nutans*

Sl. No.	Activity	In-Vitro Assay	Details	References
1.	Anti-hepatocellular activity	Pyrogallol Assay Evaluation of Antioxidant Capacity	Treatment ($\mu\text{g/ml}$): 100, Result: Inhibition of pyrogallol autoxidation by 18.7%. Cell Type: HepG2, Treatment ($\mu\text{g/ml}$): 25, 50, 100, Time (h): 1, Result: Inhibited H ₂ O ₂ -induced ROS.	[13]
2.	Antimicrobial Activity		Microorganism: <i>E. coli</i> , <i>S. aureus</i> , Treatment ($\mu\text{g/ml}$): 0.25, 0.12, Time (h): 24, Result: Presence of dead cells. Microorganism: <i>A. fumigatus</i> , Treatment ($\mu\text{g/ml}$): 0.25, Result: Higher zone of inhibition compared to other fungi.	[28]
3.	Anti-diabetic activity	α Glucosidase Activity Assay	Treatment ($\mu\text{g/ml}$): 0.1, Result: Inhibitory effects in decreasing order: ethyl acetate > hexane > butanol > aqueous > methanol fraction of the extract.	[28]
4.	In-vitro Anti-inflammatory Assay	Hypotonicity Induced HRBC Membrane Stabilization Test Protein Denaturation Test	Inhibited haemolysis of HRBC, but was significantly less effective than diclofenac. Inhibited denaturation of egg albumin, but was significantly less effective than diclofenac.	[63]
5.	Anti-oxidant activity	DPPH Antioxidant Assay ABTS Assay FRAP Assay Reducing Power Assay Ferrous Ion Chelating Activity DPPH, ABTS, FRAP, TAC Assays	West Sikkim flower extracts had the highest DPPH activity at $78.90 \pm 0.98 \mu\text{g/ml}$. The root extract's ethyl acetate fraction had the highest radical scavenging activity at $21.31 \pm 2.00 \mu\text{g/ml}$. The root extract's butanol fraction showed the most potent scavenging activity at $667.63 \pm 8.90 \mu\text{g/ml}$. The root extract's 80% methanol fraction showed the greatest reducing activity at $226.66 \pm 9.50 \mu\text{g/ml}$. The root extract's ethyl acetate fraction showed the highest reducing power at $0.53 \pm 0.09 \mu\text{g/ml}$. The root extract's hexane fraction showed the highest ferrous ion chelating capacity at $77.72 \pm 6.50 \mu\text{g/ml}$. Leaf extracts with methanol were shown to be more effective than those from roots and flowers.	[6, 13, 20, 28]

Polyphenols are well-known for their antimicrobial properties, a quality often attributed to their use in traditional plant-based remedies [29].

Interestingly, the leaf extract of *Tupistra nutans* exhibits higher levels of phenols and flavonoids compared to its root and inflorescence extracts, resulting in superior antioxidant potential [20]. This observation suggests that different plant parts may contain varying concentrations of bioactive compounds, potentially influencing their therapeutic efficacy.

Surprisingly, the boiled extract of the inflorescence demonstrated higher phenolic and flavonoid content than any of the hydro-methanolic extracts, highlighting the significant impact of extraction methods on the phytochemical profile and, consequently, the antioxidant capacity of the extracts.

Flavonoids

Flavonoids, a class of polyphenolic secondary metabolites found in plants, are renowned for their antioxidant, anti-inflammatory, and anticancer properties [30, 31]. These compounds are abundant in all parts of the plant, including the leaves, roots, and flowers [20].

The leaves of *Tupistra nutans* exhibit high anticancer activity, which aligns with the known anticancer properties of flavonoids [20]. In addition to flavonoids, *T. nutans* also contains piperidine, a compound with recognized anti-cancer properties [32, 33]. This compound, exclusively found in the ETNlf extract, could contribute to the superior anticancer effect of this extract [20].

Furthermore, the boiled extract of the inflorescence was found to contain higher levels of flavonoids and exhibited greater antioxidant potential than any of the hydro-methanolic extracts. This is attributed to the release of more phenolics and flavonoids due to the loosening of plant material through heating, despite the degradation of some heat-sensitive anticancer components [20].

Given the high concentration of flavonoids and their proven anticancer properties, further in-depth studies are needed to elucidate the mechanisms underlying their cancer cell inhibition.

Table 6 Overview of phytochemicals, extraction solvents, plant parts, and their Pharmacological implications [13, 20, 28]

Sl. no.	Isolated Compounds	Solvent	Part of Plant	Pharmacological Activity	Refer-ences
1	L-Phenylalanine	80% Methanol	Root	Antibacterial activity.	[88]
2	Protocatechuic acid	80% Methanol	Root	Antibacterial, antioxidant, antidiabetic, anticancer, anti-ageing, antifibrotic, antiviral, anti-inflammatory, analgesic, antiseptic, anti-atherosclerotic anti-hyperlipidaemic, hepatoprotective, cardioprotective, nephroprotective, neuroprotective.	[64]
3	Chlorogenic acid	80% Methanol	Root	Antidiabetic and anti-obesity, antioxidant and anti-inflammatory, anti-hypertension, and anti-microbial.	[89]
4	p-Hydroxybenzoic acid	80% Methanol	Root	Antimicrobial, anti-sickling, antialgal, antioxidant antiviral, antimutagenic, estrogenic effect, hypoglycemic, nematocidal, anti-atherogenic, and antiplatelet.	[90]
5	Caffeic acid	80% Methanol	Root	Antioxidant, Anti-inflammatory, Anticancer, Neuroprotective, Anti-diabetic, Antibacterial, Antifunga.	[91]
6	p-Coumaric acid	80% Methanol	Root	Antioxidant, antimicrobial, antiviral, anti-mutagenesis, anti-cancer.	[92]
7	Ferulic acid	80% Methanol	Root	Antioxidant, anti-inflammatory, anti-fibrosis, anti-apoptotic, anti-platelet.	[93]
8	Salicylic acid	80% Methanol	Root	Antioxidant, anti-inflammatory, and antimicrobial.	[94]
9	Phenol, 2,4-bis(1,1-dimethyl ethyl)-	Methanol	Inflorescence	Antifungal, anti-virulence.	[50, 95]
10	9-Hexadecenoic acid, methyl ester, (z)-	Methanol	Inflorescence	Anti-inflammatory.	[96]
11	Hexadecenoic acid, methyl ester	Methanol	Inflorescence	Anti-bacterial, anti-cancer, Analgesic Antipyretic Anti-inflammatory Anti-fungal Antioxidant	[97–99]
12	6,9,12,15-Docosatetraenoic acid	Methanol	Inflorescence	Cardioprotective, hypo-cholesterolemic, anti-nociceptive, antioxidant	[98]
13	Linoleic acid, methyl ester	Methanol and Hydro-methanol (1:1)	Root, Leaf, Inflorescence	Anti-tubercular, antimalarial, neuroprotective, cardioprotective, anti-inflammatory, anticancer, hepatoprotective, antioxidant and anti-diabetic	[100, 101]
14	Elaidic acid, methyl ester	Methanol	Inflorescence	Inhibit cholesterol uptake, pro-inflammatory effect, anticancer, antioxidant, and anti-inflammatory	[102, 105]
15	9-Octadecenoic acid, methyl ester, (z)-	Methanol and Hydro-methanol (1:1)	Root, leaf and Inflorescence	Anti-inflammatory, anti-diarrhoea, antidiabetic, anticancer	[106]
16	Methyl stearate	Methanol	Inflorescence	LDL lowering capacity, pro-inflammatory, Nematicidal	[107, 109]
17	Eicosanoic acid, methyl ester	Methanol	Inflorescence	Antioxidant	[110, 111]
18	Glycidyl oleate	Methanol	Inflorescence	Genotoxic	[113]
19	Diisooctyl phthalate	Methanol	Inflorescence	Anti-androgenic activity, Antimicrobial, Insecticidal	[112, 113].
20	Tetracosanoic acid, methyl ester	Methanol	Inflorescence	Antidiabetic	[114]
21	Eperuic acid, (-)	Methanol	Inflorescence	Antiviral, anti-inflammatory, antioxidant, anti-tumour, and anti-hepatic steatosis	[115]
22	Vitamin E	Methanol and Hydro-methanol (1:1)	Root, leaf and Inflorescence	Antioxidant, anti-atherogenic, anti-obesity, bone and joint protection, anticancer, prevent infertility, anti-inflammatory, and neuroprotective	[116, 117]
22	Cholesterol	Methanol	Inflorescence	Precursor of steroid hormone, bile salts, and vitamin D High cholesterol cardiovascular disease (118).	[118]
23	Diosgenin	Methanol and hydro-methanol (1:1)	Root and Inflorescence	Anticancer, Cardioprotective, anti-diabetes, neuroprotective, immunomodulatory, estrogenic, and skin protective effects, anti-infectious, anti-coagulant, antioxidant, Antiatherosclerosis, Anti-asthmatic, Hepatoprotective, Anti-osteoporosis, Anti-thyroiditis	[47, 119].
24	Stigmast-5-en-3.beta.-or	Methanol and hydro-methanol (1:1)	Root, leaf and Inflorescence	Antimicrobial, Anti-inflammatory, Anticancer, Antioxidant, Angiogenic, Immunomodulatory, Antihyperlipidemic, Antiatherosclerosis, Anti-diabetes	[48].

Table 6 (continued)

Sl. no.	Isolated Compounds	Solvent	Part of Plant	Pharmacological Activity	References
25	Palmitic acid	Hydro-methanol (1:1)	Root, Leaf, Inflorescence	Anticancer, antiviral, anti-inflammatory, analgesic, and regulation of lipid metabolism	[120]
26	Piperidine	Hydro-methanol (1:1)	Leaf	Anti-cancer, anti-diabetic, Antipsychotics, HIV-1 Inhibitors, Anti-inflammatory, Anti-Alzheimer,	[121, 122]
27	Phytol	Methanol and hydro-methanol (1:1)	Leaf	Antioxidant, anticancer, antibacterial, anti-inflammatory, and antinociception, cytotoxic.	[123, 124]
28	Myo-inositol	Hydro-methanol (1:1)	Leaf	Antioxidant, cardioprotective, antidiabetic, anticancer, antidepressant, and anti-inflammation.	[125]
29	β -citronellol	Hydro-methanol (1:1)	Root and Inflorescence	Antifungal, antibacterial, anticonvulsant, analgesic, anticancer	[126, 127]
30	Neophytadiene	Hydro-methanol (1:1)	Root, leaf and inflorescence	Antimicrobial, analgesic, antipyretic, antioxidant, and anti-inflammatory, neuroprotective.	[82, 128]
31	9,12-octadecadienoyl chloride	Hydro-methanol (1:1)	Root, leaf and inflorescence	Antisecretory, choleric, and antitubercular.	[129]
32	9-octadecenoic acid	Hydro-methanol (1:1) and methanol	Root, leaf and inflorescence	Anti-inflammatory, antidiabetic and anticancer.	[106, 126, 130, 131]
33	Stearic acid	Hydro-methanol (1:1)	Root, leaf and inflorescence	Ldl reducing capacity.	[108]
34	Phthalic acid	Hydro-methanol (1:1)	Root, leaf and inflorescence	Antidiabetic, antioxidant.	[127, 132]
35	Beta tocopherol	Hydro-methanol (1:1)	Root, leaf and inflorescence	Antioxidant.	[133]
36	Gamma tocopherol	Hydro-methanol (1:1)	Root, leaf and inflorescence	Anticancer, antioxidant, cardioprotective, neuroprotective, anti-inflammation.	[134]
37	Stigmasterol	Hydro-methanol (1:1)	Root, leaf and inflorescence	Anticancer, antidiabetic, antioxidant, antibacterial, antifungal, anti-osteoarthritis, anti-inflammatory, immunomodulatory, neuroprotective, antifungal, antiparasitic, and antiviral.	[80]
38	Beta-sitosterol	Hydro-methanol (1:1)	Root, leaf and inflorescence	Antioxidant, anticancer, Angiogenic Effect, Antihyperlipidemic, Anti-Atherosclerosis, antimicrobial, antidiabetic, anti-inflammatory, antipyretic, anti-pulmonary tuberculosis, anti-arthritis, immune modulation and anti-HIV.	[135, 136]
39	Piperidine	Hydro-methanol (1:1)	Leaf	Anticancer, antimicrobial, analgesic, anti-inflammatory, antipsychotic, anticancer, and neuroprotective.	[121, 122, 137]
40	Squalene	Hydro-methanol (1:1)	Root, leaf and inflorescence	Emollient, antioxidant, antitumour, anti-inflammation, cardioprotective, and antibacterial.	[138, 139]
41	Pearlside	Hydro-methanol (1:1)	Root, leaf and inflorescence	Antitumour.	[140]
42	Alpha santalol	Hydro-methanol (1:1)	Root, leaf and inflorescence	Anticancer, antidiabetic, antipsychotic, antibacterial, sedative, antiviral, anti-psoriasis.	[141, 142]
43	Gamma sitosterol	Hydro-methanol (1:1)	Root, leaf and inflorescence	Anticancer, hypolipidemic agent.	[143, 144]

Pharmacological Activity

Anti-diabetic activity

A 2019 study identified *Tupistra nutans* as the only species within its genus to be tested for antidiabetic activity [8, 11]. The study involved extracting an 80% methanolic root extract of *T. nutans* using various solvents: n-hexane, ethyl acetate, n-butanol, methanol, and water. Among these, the ethyl acetate extract exhibited the highest α -glucosidase inhibitory effect (81.68%), suggesting its potential as an antidiabetic agent [8]. This extract also demonstrated the highest phenolic (51.20 mg/g

GAE) and flavonoid (15.20 mg/g QE) content, further underscoring its therapeutic potential [8].

Subsequent studies in 2023 revealed the presence of compounds like Phthalic acid, Stigmasterol, Diosgenin, Alpha-santalol, and Beta-sitosterol in *T. nutans*, all of which are known for their anti-diabetic properties. Additionally, the methanolic root extract was found to contain phytochemicals with potential antidiabetic properties [12]. The inflorescence portion was found to contain 9-Octadecenoic acid, methyl ester, and Tetracosanoic

acid, methyl ester, both of which have proven antidiabetic effects [12].

A significant positive correlation was observed between α -glucosidase inhibition and total phenolic content (TPC) and total flavonoid content (TFC) [8]. This suggests that phenolic compounds may be major contributors to the α -glucosidase inhibitory activity of *T. nutans* [8].

These findings collectively indicate that *T. nutans* holds promise for promoting human health, particularly in diabetes management. However, further in-depth studies are necessary to elucidate the mechanisms underlying the α -glucosidase inhibitory activity of these compounds and to mitigate potential oxidative stress at higher concentrations.

Anti-microbial activity

The root extracts of *Tupistra nutans* have been shown to possess potent antimicrobial and antifungal properties against a wide range of pathogens [28]. These extracts exhibit significant cytotoxic effects on *E. coli* and *S. aureus* at concentrations ranging from 0.25 to 0.12 mg/mL. This dose-dependent cytotoxicity, confirmed by propidium iodide staining, is accompanied by inhibition zones ranging from 10.17 ± 1.10 to 19.16 ± 1.25 mm at a concentration of 0.25 mg/mL. Interestingly, *A. fumigatus* among the tested *Aspergillus* species showed higher susceptibility, suggesting potential species-specific activity [28]. A concentration of 0.12 mg/mL was sufficient to completely inhibit the growth of most pathogens, except for *S. aureus*. This highlights the diverse susceptibility profiles of the tested organisms and the potential of *T. nutans* extracts in combating microbial infections [28].

T. nutans is rich in various bioactive compounds, including:

- Phenolic compounds: Protocatechuic acid, Ferulic acid, P-Hydroxybenzoic acid, Salicylic acid, Chlorogenic acid, P-Coumaric acid, Caffeic acid, and L-phenylalanine.
- Fatty acids: Palmitic acid, Linoleic acid, Phytol, 9-octadecanoic acid.
- Sterols: Stigmasterol, Diosgenin, β -Sitosterol.
- Other compounds: Piperidine, Vitamin E, Cholesterol, and various methyl esters.

These compounds, confirmed to possess antimicrobial properties, contribute to the overall antimicrobial activity of *T. nutans* extracts.

Further research is needed to isolate and identify individual phenolic compounds and conduct in vivo studies to better understand the antidiabetic mechanism of action of *T. nutans*. The antimicrobial properties of *T. nutans*, supported by the presence of these bioactive

compounds, make it a promising candidate for future research in the field of antimicrobial therapy.

Anti-inflammatory property

Tupistra nutans, a plant renowned for its potent antioxidant properties, has been investigated for its potential anti-inflammatory applications [28, 61, 62]. The methanolic root extract of this plant has demonstrated significant anti-inflammatory activity, attributed to the presence of bioactive metabolites, specifically flavonoids and phenolics [63]. These metabolites have been shown to inhibit human red blood cell (HRBC) membrane stabilization and protein denaturation, key processes in inflammation, in a dose-dependent manner. However, when compared to the standard drug diclofenac, *T. nutans* was found to be less effective. This statistical difference was confirmed with an F-value of 4.227, degrees of freedom (df) of 2, and a p-value of 0.033.

Further phytochemical analysis of the methanolic root extract revealed a plethora of phenolic compounds with known anti-inflammatory properties, including:

- Protocatechuic acid [64].
- Ferulic acid [65].
- Hydroxybenzoic acid [66].
- Salicylic acid [67].
- Chlorogenic acid [68].
- Coumaric acid [69].
- Caffeic acid [70].
- L-phenylalanine [71].

Additionally, a comprehensive phytochemical analysis of the methanolic root extract and inflorescence extract (METNI) identified several compounds with established anti-inflammatory properties, including:

- 9-Hexadecenoic acid, methyl ester (Z) [72].
- Linoleic acid methyl ester [73].
- Phytol [74].
- Glycidyl oleate [75].
- 9-Octadecenoic acid [4].
- 6,9,12,15-Docosatetraenoic acid, methyl ester [76].
- Cholesterol [77].
- Diosgenin [47].
- Vitamin E [78].
- Beta-sitosterol [79].
- Stigmasterol [80].
- Squalene [81].
- Neophytadine [82].
- Myo-inositol [83].
- Stearic Acid [84].
- Phthalic Acid [85].
- Piperidine [33].
- β -Tocopherol [86].

- Salicylic Acid [87].

These findings highlight the therapeutic potential of *T. nutans* in anti-inflammatory treatments. Future research should focus on elucidating the precise mechanisms of action of these compounds to better understand their anti-inflammatory effects and potential therapeutic applications.

Anti-cancer activity

Tupistra nutans, a plant species distinguished for its anti-oxidant, anti-inflammatory, and antimicrobial attributes, is currently the subject of scientific investigation for its prospective role in cancer therapy. The plant's abundant fiber content is postulated to aid in mitigating the risk of specific cancers [6], notably those of the breast and colon. The observed anticancer activity in a related species, *Tupistra chinensis* [63], further emphasizes the potential of *Tupistra nutans* in oncology.

A study involving HepG2 and F111 cell lines found that a 200-microgram concentration of the 80% methanolic root extract of *Tupistra nutans* significantly impeded cell proliferation by 59% and 31% respectively. The extract was observed to induce G0/G1 phase arrest in HepG2 cells in a concentration-dependent manner and significantly enhance the sub-G0/G1 population, indicative of its anticancer activity. In F111 cells, G0/G1 phase arrest was noted with the extract at 50, 100, and 200 micrograms, but there was no significant change in the sub-G0/G1 population. Interestingly, a 25-microgram concentration of the extract resulted in a proliferation of F111 cells [13].

The MTT assay revealed that the extract caused significantly more cell proliferation inhibition in HepG2 cells (IC₅₀=138 µg/ml) compared to F111 cells (IC₅₀=347 µg/ml). The extract also instigated the generation of intracellular Reactive Oxygen Species (ROS) and modified the cell cycle at higher concentrations [13].

In another study, the root, leaf, and flower extracts of methanolic *Tupistra nutans*, at a high concentration of 400 µg/ml, significantly curtailed cell viability in HepG2 cells. After 12 h, the root, leaf, and flower extracts resulted in cell viability reductions of 77.05%, 83.27%, and 74.36% respectively. The leaf extract demonstrated the highest capacity to decrease cell viability and inhibit HepG2 cell proliferation, with an IC₅₀ value of 34.60 µg/ml. It also increased intracellular ROS production by 2.20-fold, decreased MMP by 2.70-fold, led to the highest intracellular superoxide generation (1.54-fold), reduced mitochondrial mass (2.70-fold), and depleted GSH levels by 1.41-fold in HepG2 cells [20].

The methanolic extract derived from the root, leaf, and inflorescence (METNI) of *Tupistra nutans* has been identified to encompass a variety of bioactive compounds

[13, 20, 28]. These compounds, known for their anti-cancer properties, include Protocatechuic acid, Chlorogenic acid, Ferulic acid, Hydrobenzoic acid, Stigmasterol, Gamma tocopherol, Palmitic acid, Phytol, Piperidine, Vitamin E, Gamma sitosterol, 9-Octadecenoic acid, Alpha santalol, Citronellol, Salicylic acid, Coumaric acid, Caffeic acid, L-Phenylalanine, Hexadecenoic acid, Beta-sitosterol methyl ester, Linoleic acid methyl ester, Elaidic acid methyl ester, and Diosgenin. This discovery further underscores the potential of *Tupistra nutans* in the realm of cancer therapeutics.

These findings suggest that *Tupistra nutans* could be a potential anticancer agent, provided the dosage is carefully controlled. However, further comprehensive investigations are necessary for a more informed risk-benefit evaluation.

Anti-oxidant property

Tupistra nutans, a plant known for its antioxidant, anti-inflammatory, and antimicrobial properties, is being investigated for its potential role in cancer therapy. The plant's high fiber content is believed to contribute to reduced risk of certain cancers, particularly breast and colon cancer [6]. The anticancer activity observed in a related species, *Tupistra chinensis* [63], further supports the potential of *T. nutans* in oncology.

A study on HepG2 and F111 cell lines revealed that a 200-microgram concentration of the 80% methanolic root extract of *T. nutans* significantly inhibited cell proliferation by 59% and 31%, respectively. The extract induced G0/G1 phase arrest in HepG2 cells in a concentration-dependent manner and significantly increased the sub-G0/G1 population, indicating its anticancer activity. In F111 cells, G0/G1 phase arrest was observed at 50, 100, and 200 micrograms, but there was no significant change in the sub-G0/G1 population. Interestingly, a 25-microgram concentration of the extract led to increased proliferation of F111 cells [13].

The MTT assay showed that the extract inhibited cell proliferation more significantly in HepG2 cells (IC₅₀=138 µg/ml) compared to F111 cells (IC₅₀=347 µg/ml). The extract also induced the generation of intracellular Reactive Oxygen Species (ROS) and altered the cell cycle at higher concentrations [13].

Another study found that the root, leaf, and flower extracts of *T. nutans*, at a high concentration of 400 µg/ml, significantly reduced cell viability in HepG2 cells. After 12 h, the root, leaf, and flower extracts reduced cell viability by 77.05%, 83.27%, and 74.36%, respectively. The leaf extract demonstrated the highest capacity to inhibit HepG2 cell proliferation, with an IC₅₀ value of 34.60 µg/ml. It also increased intracellular ROS production, decreased mitochondrial membrane potential (MMP), increased intracellular superoxide generation, reduced

mitochondrial mass, and depleted GSH levels in HepG2 cells [20].

The methanolic extract of *T. nutans* root, leaf, and inflorescence (METNI) contains various bioactive compounds with known anticancer properties, including:

- Protocatechuic acid.
- Chlorogenic acid.
- Ferulic acid.
- Hydrobenzoic acid.
- Stigmasterol.
- Gamma-tocopherol.
- Palmitic acid.
- Phytol.
- Piperidine.
- Vitamin E.
- Gamma-sitosterol.
- 9-Octadecenoic acid.
- Alpha-santalol.
- Citronellol.
- Salicylic acid.
- Coumaric acid.
- Caffeic acid.
- L-Phenylalanine.
- Hexadecenoic acid.
- Beta-sitosterol methyl ester.
- Linoleic acid methyl ester.
- Elaidic acid methyl ester.
- Diosgenin.

These findings suggest that *T. nutans* has potential as an anticancer agent, but careful dosage control is crucial. Further comprehensive studies are needed to fully understand its mechanisms of action and potential side effects.

Conclusion

In summary, *Tupistra nutans*, a member of the Asparagaceae family, has a rich ethnobotanical history, particularly its floral parts, which are consumed as a curry for their purported antidiabetic properties. Scientific investigations have supported these traditional uses, revealing a range of pharmacological activities, including antioxidant, anti-inflammatory, antidiabetic, antimicrobial, and anti-hepatocellular carcinoma properties. However, a significant limitation in our current understanding of the plant's medicinal potential lies in the absence of in vivo studies.

Future research should prioritize in vivo experiments to establish the therapeutic efficacy and safety profile of *T. nutans* in living systems. Additionally, identifying and characterizing the active phytochemicals, understanding their mechanisms of action, and assessing the impact of agricultural practices on the plant's medicinal properties are crucial next steps. Sustainable harvesting practices

are essential to preserve *T. nutans* for future therapeutic use and research.

Abbreviations

cm	Centimetre
mm	Millimetre
UFAs	Unsaturated Fatty Acids
SFAs	Saturated Fatty Acids
LDL	Low-Density Lipoprotein
HDL	High-Density Lipoprotein
CVD	Cardiovascular Disease
METNI	Methanolic extracts of <i>Tupistra nutans</i> inflorescence
GC-MS	Gas Chromatography–Mass Spectrometry
HepG2	Human liver cancer cell line
F111	Cell line derived from a rat embryo
NF-κB	Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
STAT3	Signal Transducer and Activator of Transcription 3
ROS	Reactive Oxygen Species
ETN _L	Hydro-methanolic extracts of leaf of <i>tupistra nutans</i>
GAE	Gallic Acid Equivalents
mg	Milligram
g	Gram
QE	Quercetin Equivalents
TPC	Total Phenolic Content
TFC	Total Flavonoid Content
mL	Millilitre
<i>E. coli</i>	<i>Escherichia coli</i>
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
<i>A. fumigatus</i>	<i>Aspergillus fumigatus</i>
HRBC	Human Red Blood Cells
df	Degrees of Freedom
G0 phase	Resting phase
G1 phase	First gap phase
μg	Microgram
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
IC50	Half Maximal Inhibitory Concentration
GSH	Glutathione
DPPH	2,2-diphenyl-1-picrylhydrazyl
ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
FRAP	Ferric Reducing Ability of Plasma
TAC	Total Antioxidant Capacity
H2O2	Hydrogen Peroxide
h	Hour
F	F-statistic
p	P-value
MMP	Matrix Metalloproteinase

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Data availability

No datasets were generated or analysed during the current study.

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