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Mosquitocidal, Antioxidant activity and Cytotoxicity of *Tarenna alpestris:* A medicinal plant from Megamalai Hills, Theni, Tamil Nadu- India

Chinnappan Sagayaraj, Uthaman Danya*
Department of Botany, Nirmala College for Women, Redfields, Coimbatore, Tamil Nadu, India

Abstract
Mosquito-borne diseases are illnesses that spread to people by the bite of an infected mosquito. These illnesses are major public health concerns worldwide due to their high prevalence, impact on quality of life, and potential for severe outbreaks. The dengue virus causes the disease, which is carried by Aedes mosquitos, mainly *Aedes aegypti*. Dengue fever frequently causes a high fever, severe headache, pain behind the eyes, joint and muscular pain, and rash. In severe situations, it can cause dengue hemorrhagic fever and dengue shock syndrome. *Tarenna alpestris*, a lesser-known medicinal plant found in the Megamalai Hills of Theni, Tamil Nadu, India, has attracted attention due to its possible therapeutic effects. The purpose of this study is to analyze *Tarenna alpestris*' pharmacological potential by evaluating its mosquitocidal, cytotoxic, antioxidant, and efficacious properties. The plant extracts were produced with hexane solvent and subjected to a variety of tests. To investigate the plant's potential as an anti-cancer agent, cytotoxicity was measured using the MTT assay on colon cancer cells HT29. Antioxidant activity was assessed using DPPH, ABTS, and FRAP tests to determine the ability to neutralize free radicals. *Tarenna alpestris* was found to have considerable cytotoxic effects, namely inhibiting cancer cell proliferation. Its antioxidant activity was high, with strong radical scavenging properties. These findings imply that *Tarenna alpestris* has promising therapeutic potential, which supports its usage in traditional medicine and calls for additional research into its bioactive components and mechanisms of action.

Keywords: *Aedes aegypti*, preliminary phytochemicals, medicinal plant, pharmacology, Cytotoxicity, microbial pathogens

Introduction

Mosquito-borne diseases are illnesses transmitted to humans through the bite of infected mosquitoes. Caused by Plasmodium parasites and transmitted by Anopheles mosquitoes, it results in fever, chills, and flu-like symptoms. Transmitted by Aedes mosquitoes, it causes high fever, severe headaches, pain behind the eyes, and joint and muscle pain. Severe cases can lead to dengue hemorrhagic fever or dengue shock syndrome. Spread by Aedes mosquitoes, it often causes mild symptoms such as rash and joint pain but is particularly concerning for pregnant women due to the risk of birth defects. Also transmitted by Aedes mosquitoes, it leads to high fever and severe joint pain, which can be long-lasting. Transmitted by Culex mosquitoes, it can range from mild flu-like symptoms to severe neurological conditions [1].

Dengue is endemic in many parts of India, with outbreaks occurring during the monsoon season. The disease can range from mild to severe, with severe cases potentially leading to dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS), which can be life-threatening. High rates of hospitalization and mortality can occur during severe outbreaks, placing a strain on the healthcare system. Severe dengue cases require intensive medical care, which can be a challenge in resource-limited settings. Common symptoms include high fever, severe headache, pain behind the eyes, joint and muscle pain, and rash. In severe cases, complications such as bleeding, plasma leakage, and organ damage can occur. Efforts to control mosquito populations, such as larviciding, fogging, and eliminating breeding sites, are crucial in managing dengue. Community participation is essential for effective control [2].

Plants are a popular source of nutrients and nutraceuticals due to their potential health benefits and medicinal properties. Herbal therapy is used by over 80% of the rural population. Natural compounds present in plants are employed as alternative medicines and play a major role in people's health and well-being all over the world. Many commonly used medications, such as atropine, reserpine, digoxin, tubocurarine, morphine, ephedrine, quinine, and aspirin, were identified from medicinal plants. The hydroxyl groups in phenols and flavonoids contribute to them being an appealing group of phytoconstituents for their potential to quench singlet and triplet oxygen, breakdown peroxides, and heal oxidative damage [3, 4].

Endogenous mechanisms in mammalian cells generate free radicals, commonly known as reactive oxygen species (ROS). An imbalance in the ratio of ROS production to detoxification causes oxidative stress, which can disrupt physiological processes, resulting in chain reactions in lipid membranes and damage to DNA-repairing enzymes. Phenols and flavonoids have been shown to prevent or delay cancer by decreasing oxidative damage and improving RNA and DNA repair (Vance *et al*., 2016). As a result, it is critical to develop alternative medicine for the treatment and prevention of diseases by searching for plants rich in phenols and flavonoids [4].

In contrast, medicinal plants contain both medicinally beneficial and possibly hazardous chemicals. Cytotoxicity assays are used to predict possible toxicity in normal or altered cultured cells. Prior to conducting safety studies on complete organisms, cultured cells are often exposed to test compounds for a brief period of time to discover how the chemical may alter fundamental or specialized cell processes. Furthermore, it can give information on the carcinogenic and genotoxic effects of herbal-derived chemicals and extracts [5].

In this present study, we assess the mosquitocidal activity of *Aedes aegypti* using *T. alpestris*. HE (Hexane extract) using radical scavenging assays such as DPPH, ABTS and FRAP. Finally, we evaluate the cytotoxic effects of *T. alpestris* extracts on human colon cancer cell lines HT29.

Materials and methods

DPPH free radial scavenging activity

The goal of this study is to determine the efficacy of botanical extracts in neutralizing DPPH radicals. The aforementioned extract has the potential to donate hydrogen or scavenge radicals, resulting in the conversion of the 2,2-diphenyl-1-picrylhydrazyl radical into 2,2-diphenyl-1-picrylhydrazine. This transformation causes the solution to have a faint yellow tint. The mixture is then incubated for 20 minutes at a temperature of 27 degrees Celsius. A test tube containing 100 µL of methanol and 5 mL of DPPH solution served as a negative control in the experiment. The inhibition can be evaluated by evaluating the decrease in the amount of the purple hue produced by the DPPH solution, resulting in a transition to a pale yellow hue. The difference in absorbance values between the sample and the negative control is used to calculate percentage inhibition, which is then factored into the equation below [6].

% Inhibition = [(Control OD - Sample OD)/ Control OD] x 100

ABTS·+ Scavenging Activity

To determine the overall antioxidant capacity of plant extracts, we will utilize the ABTS•+ assay for radical cation 2,2′-azinobis (3-ethylebenzothiozoline-6-sulphonic acid). The blue/green ABTS chromophore is synthesized through a chemical reaction between ABTS and potassium persulfate. This approach produces the ABTS radical directly. Following the addition of antioxidants, the radical cation is converted to ABTS, causing the solution's color to shift. The operation lasted 12 to 16 hours at ambient temperature and without lights. Under equilibrium, this solution's absorbance at 734 nm should be 0.7 ± 0.02. To carry out the experiment, varied doses of the medication are added to one microliter of diluted ABTS solution. This procedure involves diluting Trolox standards with an equal volume of ABTS solution dissolved in ethanol. The solution's ultimate concentration should range from 0 to 15 µM. To create a blank, ethanol is dissolved in ABTS, serving as the negative control while trolox is used as the reference standard. The results were expressed as milligrams of trolox equivalents per gram of pure leaf extract.

Scavenging activity % = [(Control OD - Sample OD)/ Control OD] x 100

Ferric Reducing Antioxidant Power (FRAP) Assay

Using this approach, a ferric-tripyridyl-s-triazine complex can be reduced to its ferrous form, yielding a violet-colored liquid. Synthetic chemicals including TPTZ, hydrochloric acid, acetic acid, and sodium acetate are used. To produce a solution, combine TPTZ (20 mM) with 40 mM HCl, FeCl3 (20 mM), and 25 mL of 0.2 M acetate buffer (pH 3.6). The acetate buffer contains sodium acetate and acetic acid as components. Continue using these steps to incorporate the fix. c, a, b. Steam the solution for 30 minutes at 37°C. It is critical to utilize 5 mM FeSO4•7 hypertonic acid as a reference. To guarantee that the quantity of solution in each test container is consistent, make three duplicates of the sample and distil water. After fully mixing 900 liters of FRAP solution, allow it to settle at 37 degrees Celsius for 30 minutes. After incubation, quantify the mixture's absorption of the 593 nm chromophore. The FRAP number is defined as millimoles of Fe (II) expressed per milligram of extract. The values were expressed as milligrams of trolox equivalents per gram of pure HE-*T. alpestris* (mg TE/g), with trolox as the standard.

[(Control OD - Sample OD)/ Control OD] x 100

Larvicidal/ pupicidal activity of *T. alpestris* toxicity assay of *Ae. aegypti* in standard laboratory condition

According to the methods described in Panneerselvam *et al*. [7], 25 *Ae. aegypti* larvae or pupae (first, second, third, and fourth) were kept for 24 hours in a glass container with 250ml of distilled water, along with the ideal grouping of *T. alpestris* extract at different concentrations (100ppm-500ppm). Every measured concentration yielded 0.5 mg of larval feed. Each experiment was carried out many times on all pupae and instars. The following equation was used to calculate the fatality rate.

$$\% \text{ mortality} = \frac{\text{No. of individuals dead}}{\text{No. of individuals treated}} \times 100$$

Evaluation of cytotoxicity

A cell cytotoxicity test [MTT, Hi-Media] was used to determine the inhibitory concentration (IC50). HT29 cells were grown in a 96-well plate (1×104 cells/well) for 48 hours until they reached 80% confluence. The culture medium was changed with HE-*T. alpestris* including fresh medium at various doses (25, 50, or 100 µg/ml), and they were treated with HT29 cells, followed by incubation for 48 hours. After removing the culture medium, 100µL of MTT solution was added to the wells and incubated at 37ºC for 4 hours. The supernatant was then removed. The formazan crystals were dispersed by adding 50 µL of DMSO to each well and incubating for 15 minutes. The optical density was measured at 620 nm with an ELISA multiwell plate reader (Thermo Multiskan EX, USA). The OD value was used to calculate the percentage of viability using the algorithm shown below.

% of viability = OD value of experimental sample/OD value of experimental control ×100.

Morphological Study

HT29 cells (1x105) were treated with HE-*T. alpestris* at various doses (25, 50, and 100 µg/mL) for 24 hours before being fixed in acetic acid:ethanol (1:3; v/v) solution. The cover slips were carefully placed on glass slides for the morphological examination. Each experimental group received three monolayer micrographs. The morphological alterations of the cells were evaluated at 40X magnification with a Nikon (Japan) bright field inverted light microscope.

Statistical analysis

The Sigma Stat statistical tool (Version 3.1) was used to analyze all of the investigated parameters. The inhibitory zones were calculated as mean ± standard deviation. Statistical significance was determined using one-way ANOVA with $P < 0.05$ and post-hoc Fischer analysis.

Results and discussion

Antioxidant properties of HE- *T. alpestris*

DPPH free radical scavenging activity

The use of DPPH radical scavenging experiments is common in the evaluation of antioxidant properties. In 2008, Qian and colleagues revealed that a certain antioxidant chemical has the capacity to effectively scavenge DPPH radicals, potentially preventing one of the multiple mechanisms by which lipid peroxidation induces oxidative stress. In our experiment, hexane extract *T. alpestris* significantly reduced ($p<0.05$) DPPH levels, indicating its radical-scavenging properties (Figure 1). The investigations revealed inhibition percentages of 45.26%, 54.87%, 61.75%, 72.45%, and 80.46%, respectively. The % inhibition showed a positive association with extract concentrations, indicating a scavenging function that is dose dependent. The found IC50 value indicates that *T. alpestris* has strong antioxidant action, however not as effective as rutin. This activity is due to the presence of polyphenolic chemicals in the extract, which are known to have radical scavenging activities. The efficiency of *T. alpestris* could be used to create natural antioxidant supplements [8]. Further research should focus on isolating and identifying the active chemicals responsible for this activity, as well as thoroughly investigating their processes.

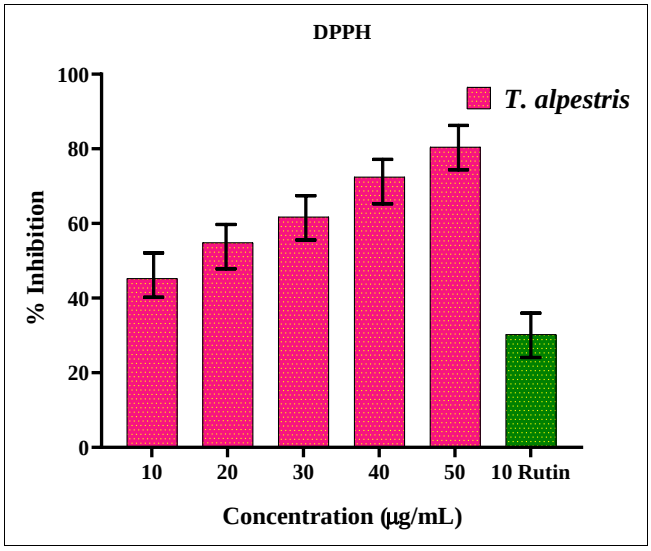

Fig 1: DPPH free radical scavenging activity

ABTS+ scavenging assay

Increasing the quantity of *T. alpestris* hexane extract resulted in a significant increase ($p<0.05$) in its ability to scavenge ABTS radicals (see Figure 2). At a trolox concentration of 34.23%, the scavenging percentages of reducing agents range from 33.25% to 68.34%. Numerous studies have shown that certain plant extracts have enhanced levels of overall antioxidant activity due to their high phenolic content. The action could be ascribed to a variety of bioactive chemicals found in *T. alpestris*, including flavonoids and phenolic acids, which are renowned for their antioxidant capabilities. The % suppression of ABTS+ radicals by the plant extract at different doses demonstrates its dose-dependent antioxidant activity. The higher the percentage inhibition at lower concentrations, the more potent the extract is as an antioxidant [9].

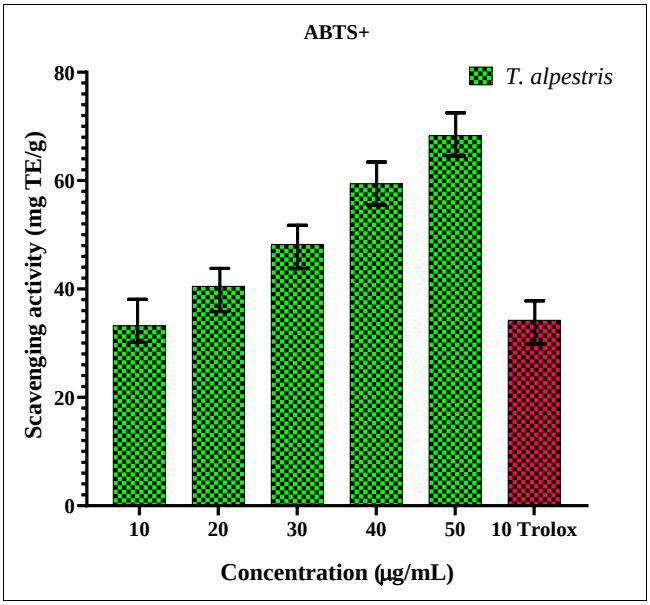

Fig 2: ABTS+ scavenging activity

FRAP antioxidant assay

The ferric (Fe3+) reducing power test converts Fe3+ to Fe2+ using a *T. alpestris* leaf extract. The procedure described above results in the formation of a ferro-ferric compound. The color of *T. alpestris* leaf extract varies according on the antioxidant concentration. The observed color changes demonstrate the extract's capacity to effectively decrease substances. The ability of a species to undergo reduction reactions can be an accurate indicator of its antioxidant activity. At 50 µg/mL, Fe3+ decreased by the maximum observed value of 63.23%. Furthermore, while testing the same quantity, a drop of 32.55% was seen in the standard trolox (refer to Figure 3). The leaf extract obtained from *T. alpestris* extract was found to have reducing power, indicating antioxidant properties. The extract has greater FRAP values, which indicates that the plant extract has a significant ability to decrease ferric ions, exhibiting significant antioxidant activity. The antioxidant activity detected in the FRAP experiment is frequently attributed to specific components in the plant extract. The current study's findings imply that the *T. alpestris* extract has significant antioxidant effects, perhaps decreasing the formation of excess free radicals [10].

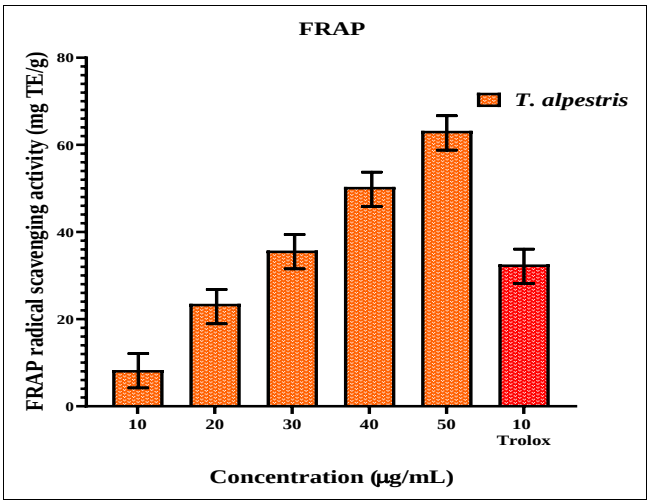

Fig 3: FRAP antioxidant activity

Larval pupal toxicity of *T. alpestris* extract

Even at modest doses, the *T. alpestris* extract proved harmful to *Aedes aegypti* larvae and pupae, the dengue vector, under typical laboratory settings. The LC50 values (ppm) for I to IV instar larvae were 226.373 µg/ml, 244.535 µg/ml, 276.603 µg/ml, and 316.635 µg/ml, respectively, while it was increased to 366.816 µg/ml for pupae (Table 1).

Similarly, low doses of *Chaetomorpha aerea* extract were harmful to *Aedes aegypti* larvae and pupae, the dengue vector, under typical laboratory conditions. The LC50 values (ppm) for I to IV instar larvae were 222.942ppm, 241.011ppm, 287.040ppm, and 316.367ppm, respectively, whereas it climbed to 349.877ppm for pupae [11].

Table 1: Larvicidal and pupicidal toxicity of *T. alpestris* against dengue vector, *Ae. aegypti.*

Mosquito life stages	LC_{50} (LC_{90}) (ppm)	95% confidence Limit LC_{50} (LC_{90})		Regression equation	χ^2 (*df*=4)
		LCL	UCL		
1st Instar	226.373 (426.458)	160.424 (360.833)	277.544 (562.118)	y = -1.450+0.006x	7.249 *n.s*
2nd Instar	244.535 (491.470)	218.554 (452.497)	268.109 (544.686)	y = -1.269+0.005x	1.374 *n.s*
3rd Instar	276.603 (550.990)	249.833 (503.518)	302.078 (617.957)	y = -1.292+0.005x	0.865 *n.s*
4th Instar	316.635 (645.924)	286.577 (578.736)	347.918 (747.714)	y = -1.232+0.004x	1.952 *n.s*
Pupa	366.816 (733.080)	333.366 (645.720)	407.054 (873.097)	y = -1.283+0.003x	2.365 *n.s*

Mortality rates are means ± SD of five replicates
No mortality was observed in the control
LC_{50}=lethal concentration that kills 50% of the exposed organisms
LC_{90}=lethal concentration that kills 90 % of the exposed organisms
χ^2 = *chi*-square value, *n.s.* = not significant (α=0.05) level

Morphological damage assessment of *Ae. aegypti* 3rd instar larvae after the treatment of *T. alpestris* extract

In this investigation, we used a light microscope to analyze *Ae. aegypti* larvae in their third instar after treatment with *T. alpestris* extract. We saw malformations including swelling, lesions, and disintegration of bodily portions. Changes in the shape or structure of the head, mouthparts, or antennae. Abnormal pigmentation and discolouration indicate injury. We see numerous ruptures and hemorrhages in bodily tissues (Figure 4).

Fig 4: Morphological damage of *Aedes aegypti* after the treatment of *T. alpestris* extract

Anticancer activity of HE- *T. alpestris*
Cytotoxic Assay

Table 2: MTT Assay

Cytotoxic activity of sample (µg/ml)	
Sample	**(Inhibitory Concentration/ IC50) HT29**
1. Extract	38µg/ml
2. Dox (STD)	19µg/ml

The effect of HE-*T. alpestris* on HT29 cells was assessed using MTT assays. HE-*T. alpestris* was tested for cytotoxicity effects on colon cancer cell lines HT29 using MTT bioassay over a 24-hour period (Fig. 5), and the IC50 value is presented in Table 2. Figure 5 reveals that HE-*T. alpestris* can suppress the proliferation of colon cancer cell lines HT29 at specific doses, with IC50 values of 38 µg/ml and 19 µg/ml for doxorubicin (standard). HE-*T. alpestris* demonstrated greater inhibitory action against cell proliferation than colon cancer cell lines HT29. The extract may cause programmed cell death, resulting in diminished cell viability. HE-*T. alpestris* may disrupt cell cycle progression, inhibiting cells from dividing and multiplying (Jaber, 2024). The extract may interfere with essential metabolic pathways necessary for cell survival and growth. The findings suggest that HE-*T. alpestris* has promise for further development as an anticancer agent [12].

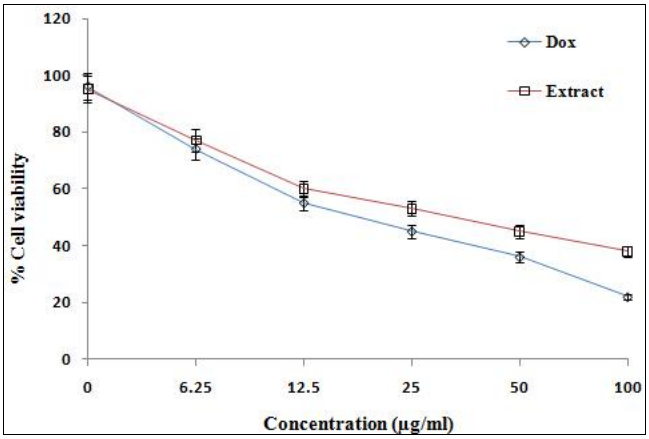

Fig 5: Cytotoxicity by MTT assay

Figure 6 depicts the morphological alterations observed in colon cancer cell lines HT29 after 24 hours of treatment with the HE-*T. alpestris* IC50 concentration. Treatment has a dose-dependent effect on the shape of the HT29 colon cancer cell line. Enhanced HE- *T. alpestris* enhanced cytotoxicity. This extract caused cell shrinkage, cell adjustment, and a reduction in the number of feasible cells. These progressions show that HE-*T. alpestris* induced apoptosis in colon cancer cell lines HT29 (Fig 6b, c, and d). In contrast, the untreated control cells showed no significant effect (Fig 6a). The morphological changes suggest that HE-*T. alpestris* causes apoptosis in HT29 cells, either through

caspase activation or the production of oxidative stress. In comparison, the treated cells displayed more severe morphological damage than the control cells and were similar to the positive control, showing that the extract has powerful anticancer action [13].

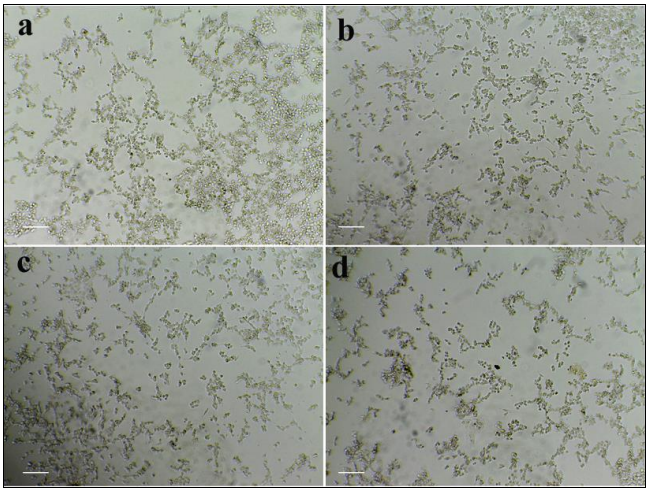

Fig 6: Morphological Assessment of HT29 cells before and after the treatment of *T. alpestris* (a) control, (b) 25 µg/mL, (c) 50µg/mL, (d) 100 µg/mL.

Conclusion

In conclusion, the study on *T. alpestris* from the Megamalai Hills has highlighted its significant potential as a medicinal plant with notable biological activities. The research demonstrates that this plant exhibits considerable mosquitocidal activity, effectively contributing to vector control, which is crucial for mitigating mosquito-borne diseases. Additionally, *T. alpestris* has shown strong antioxidant properties, indicating its potential for combating oxidative stress and related pathologies. Moreover, the cytotoxicity assessments suggest that while *T. alpestris* possesses bioactive compounds that could be harnessed for therapeutic purposes, careful consideration of its safety profile is essential. These findings underscore the value of *T. alpestris* as a source of natural products with pharmacological potential, warranting further exploration and development to fully understand and utilize its therapeutic benefits while ensuring safety and efficacy.

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