• Review

A critical overview on *Thymus daenensis* Celak.: phytochemical and pharmacological investigations

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**ABSTRACT**

*Thymus daenensis* Celak. is an herb endemic to Iran belonging to the Lamiaceae family. Growing in many parts of Iran, the plant is extensively used in folk medicine. This review was performed to compile phytochemical and pharmacological data of *T. daenensis*. Databases such as PubMed, Scopus, Web of Science, ScienceDirect, Scientific Information Database, Embase, IranMedex and Google Scholar were searched for the terms “*Thymus daenensis*” and “*Avishan-e-denaii*” up to 1st January 2014. Following reported ethnopharmacological uses, various *T. daenensis* preparations have been investigated for antimicrobial, antioxidant, insecticidal and immunomodulatory effects in recent studies. Moreover, numerous studies have been published on the composition of the herb’s essential oil, focusing either on environmental parameters or preparation methods. Due to its high concentration of thymol, the plant’s essential oil possesses high antimicrobial activities on human pathogenic strains. However, comprehensive studies on the toxicity and teratogenicity as well as clinical efficacy of *Thymus daenensis* are missing.

**Keywords:** *Thymus daenensis*; oils, volatile; plant extracts; review

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1 Introduction

Within the Lamiaceae, a family with more than 230 genera¹⁰, *Thymus* is known for many pharmacological and clinical properties. The genus *Thymus* (commonly known as Thyme) comprises hardy herbs and can adapt to extreme climatic conditions. Hence, it is widely distributed throughout the world¹⁰; more than 300 different perennial and subshrub species are mostly distributed in Europe, Asia and North Africa¹².

Known as “Avishan” in Persian, *Thymus* encompasses 14 species in the Iranian flora, four of those are endemic¹¹⁵. In Iran, leaves and flowering parts of Thyme plants are traditionally used for various medical purposes, *e.g.*, as an antispasmodic, antitussive, expectorant, carminative, anti-inflammatory or tonic agent¹⁶. Additionally, thyme is used as an herbal tea and as a spice in cooking¹⁸.

*Thymus daenensis* Celak. is one of the species of *Thymus* that is endemic to Iran. *T. daenensis* includes two identified subspecies: *T. daenensis* subsp. *daenensis* and *T. daenensis* subsp. *lancifolius*¹⁷. *T. daenensis* is a small (6–30 cm high) perennial shrub with lanceolate leaves, distributed in...
most parts of Iran, particularly over the Zagros and some parts of the Alborz mountain ranges. Its chemical composition and pharmacological activities are believed to be similar to those of Thymus vulgaris L. and the native Iranian plant, Zataria multiflora Boiss., known as Shiraz thyme. Therefore, many of the medicinal properties of thyme are attributed to T. daenensis as well. Like in all officinal species of Thymus, the most abundant active ingredient of T. daenensis is the essential oil.

Despite the abundance of T. daenensis in Iran and its extensive use in folk medicine, phytochemical analysis and pharmacological evaluation of this herb have been mostly confined to the analysis of the essential oil. Additionally, due to the hybridization of T. daenensis with different Thymus species in Iran, the herb shows high morphological diversity. In this regard, the current work was performed to outline and compile published assessments on T. daenensis and to clarify further approaches and scopes of investigation.

2 Methods of the study

The databases PubMed, Scopus, Web of Science, ScienceDirect, Scientific Information Database (Iranian database which contains both English and Persian articles), Embase, IranMedex, and Google Scholar were searched for the terms “Thymus daenensis” and “Avishan-e-denaii” up to 1st January 2014. Articles related to agricultural science were excluded. Derived information was then categorized and presented in two groups as phytochemical and pharmacological findings.

3 Results

In total, 80 papers on T. daenensis were found. Among those, 17 papers were related to genetic and agricultural science, and were excluded from the present literature review.

3.1 Phytochemical composition

3.1.1 Essential oil

Approximately 35 papers deal with the analysis of T. daenensis essential oil. This literature demonstrates that the location of collection, climate, time and type of harvesting, method of preserving and drying, as well as mode of extraction each profoundly affect the essential oil profile of T. daenensis.

The majority of the constituents identified in T. daenensis essential oil extracts are oxygenated monoterpenes. In numerous studies, thymol was quantified as the main constituent, and ranged from 4.2% to 85.2%. Thymol content in the essential oil is maximized (at ~85%) by harvesting the herb between the beginning and middle of its flowering season. However, in one investigation thymol was not detected in the studied sample. Thymol’s isomer, carvacrol, was also reported to be among the most abundant constituents in five studies. Less frequently, geraniol, geranyl acetate, borneol and 1,8-cineole were reported as main components. One study also cited linalool as the major compound of T. daenensis.

Major sesquiterpenes in T. daenensis included β-caryophyllene, α-humulene and allo-aromadendrene. Based on gas chromatography-mass spectrometer data, the main sesquiterpenes were found to be similar among different T. daenensis populations, but relative quantities varied. Table 1 represents the most reported essential oil constituents of different T. daenensis samples from Iran.

Concurrent with the phenological cycle, the essential oil profile of T. daenensis changes. In addition, many intrinsic and extrinsic factors affect the T. daenensis essential oil yield and constituents. Genetic variability and cultivation location as well as drying temperature and extraction modes are important factors. It was shown that the yield of thymol and carvacrol may be increased after drying of the plant material at higher temperature (microwave and oven 70 °C), whereas drying time had less impact on T. daenensis essential oil profile. In contrast, another study revealed that drying T. daenensis leaves below 30 °C resulted in the highest amounts of essential oil. In the employed sample, allo-aromadendrene was detected at its highest amount when samples were dried at 30 °C, while β-cymene and γ-terpinene yields were highest after drying in the shade, below 30 °C.

3.1.2 Non-volatile compounds

The presence of tannins and saponins has been demonstrated in the aerial parts of T. daenensis. In a study of the total phenolic content of T. daenensis collected at different harvest times, using the gallic acid equivalence method (GAE), phenolic quantities ranged from 18.82 to 18.97 mg GAE/g dry weight for low-flowering to full-flowering stages. Interestingly, exactly the same amounts of total phenolics at the same flowering stages were reported in another study. In a T. daenensis hydroalcoholic extract, determination of the phenolic content, using Folin-Ciocalteu’s reagent, found (295.9 ± 34.1) μg rutin equivalents/mg extract. In the same study, the flavonoid content was determined, using AlCl3 reagent, to be (35.2 ± 2.5) μg rutin/mg extract. Polar and non-polar subfractions of a T. daenensis methanol extract contained higher amounts of total flavonoids. Results of another study showed that the total phenol content of T. daenensis ((644.1 ± 6.8) μg GAE/mg extract) was much higher than that of a commercial thyme sample ((16.9 ± 2.6) μg GAE/mg extract). In another assessment, the total phenol and
Table 1  Reported major constituents of *Thymus daenensis* essential oil from Iran

<table>
<thead>
<tr>
<th>Sample</th>
<th>Major essential oil constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Thymol (74.7%)</td>
<td>ρ-Cymene (6.5%)</td>
</tr>
<tr>
<td>2 Carvacrol(17.4%)</td>
<td>α-Terpinol (13.2%)</td>
</tr>
<tr>
<td>3 Thymol (54.7%)</td>
<td>γ-Terpinene (12.9%)</td>
</tr>
<tr>
<td>4 Carvacrol (37.0%)</td>
<td>Thymol (12.8%)</td>
</tr>
<tr>
<td>5 Thymol (77.7%)</td>
<td>γ-Terpinene (5.1%)</td>
</tr>
<tr>
<td>6 Thymol (73.9%)</td>
<td>Carvacrol (6.7%)</td>
</tr>
<tr>
<td>7 Thymol (54.7%)</td>
<td>γ-Terpinene (12.9%)</td>
</tr>
<tr>
<td>8 Thymol (67.2%)</td>
<td>γ-Terpinene (5.5%)</td>
</tr>
<tr>
<td>9 Thymol (67.1%)</td>
<td>ρ-Cymene (4.9%)</td>
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<tr>
<td>10 Thymol (73.6%)</td>
<td>ρ-Cymene (9.0%)</td>
</tr>
<tr>
<td>11 Thymol (73.7%)</td>
<td>Caryophyllene (5.6%)</td>
</tr>
<tr>
<td>12 Thymol (49.7%)</td>
<td>Carvacrol (15.2%)</td>
</tr>
<tr>
<td>13 Thymol (71.5%)</td>
<td>ρ-Cymene (7.1%)</td>
</tr>
<tr>
<td>14 Thymol (72.2%)</td>
<td>ρ-Cymene (4.8%)</td>
</tr>
<tr>
<td>15 Carvacrol (80.1%)</td>
<td>Thymol (3.8%)</td>
</tr>
<tr>
<td>16 Geraniol (75.7%)</td>
<td>Geranyl acetate (8.8%)</td>
</tr>
<tr>
<td>17 Carvacrol (52.3%)</td>
<td>Thymol (16.4%)</td>
</tr>
<tr>
<td>18 Geraniol (37.2%)</td>
<td>Geranyl acetate (18.74%)</td>
</tr>
<tr>
<td>19 Thymol (74.61%)</td>
<td>ρ-Cymene (4.6%)</td>
</tr>
<tr>
<td>20 Carvacrol (28.8%)</td>
<td>Thymol (27.4%)</td>
</tr>
<tr>
<td>21 Thymol (78.3%)</td>
<td>Caryophyllene (4.3%)</td>
</tr>
<tr>
<td>22 Geraniol (66.8%)</td>
<td>Geranyl acetate (13.9%)</td>
</tr>
</tbody>
</table>

—: not presented.
total flavonoid contents of T. daenensis hydroalcoholic extract were determined to be (97.7 ± 11.6) mg GAE/g extract and (37.5 ± 2.8) mg rutin/g extract, respectively.[46]

The quantification of rosmarinic acid in T. daenensis was found to be (14.3±0.4) mg/g, using high-performance liquid chromatography.[41]

3.2 Pharmacological activities

3.2.1 Antibacterial and antifungal properties

Due to the considerable amounts of oxygenated monoterpenes, in particular thymol and carvacrol,[34,35], T. daenensis exhibits significant antimicrobial activities.[42]

In studies against the food-borne pathogen Listeria monocytogenes the essential oil was proven to be quite effective[45], whereas the activity of a hydroalcoholic extract was negligible (minimum inhibitory concentration (MIC) = 8 mg/mL).[44]. The negative result of the latter study might be explained by the lack of thymol and carvacrol in the hydroalcoholic extract.

Another study assessed the antimicrobial activity of T. daenensis essential oil against two Gram-positive and Gram-negative bacteria as well as three fungi. The essential oil was proven to be quite effective[45], whereas the activity of a hydroalcoholic extract was negligible (minimum inhibitory concentration (MIC) = 8 mg/mL).[44]. The negative result of the latter study might be explained by the lack of thymol and carvacrol in the hydroalcoholic extract.

In an investigation of an important fish pathogen, Streptococcus iniae, T. daenensis essential oil showed only very weak activity (MIC = 312 µg/mL).[45]. The hydrodistilled oil and an 80% ethanol extract of T. daenensis were also examined against the human pathogens S. aureus, E. coli, Pseudomonas aeruginosa and Klebsiella pneumonia, using agar disc diffusion and serial dilution assays. Results showed that both the essential oil and the ethanol extract, were active against P. aeruginosa (MIC = 39 µg/mL for both). The essential oil was also active against E. coli and K. pneumoniae (MIC = 39 µg/mL). In contrast, the activity of the ethanol extract against the two latter bacteria remained very low (MIC >50% = 156.25 and 625 µg/mL, respectively). Neither the essential oil nor the ethanol extract was active against S. aureus.[46].

In a comparative study, the antimicrobial activity of T. daenensis essential oil was assessed on different microbial strains. The MICs against C. albicans, E. coli and S. aureus were 40, 80 and 160 µg/mL, respectively.[39]. Against P. aeruginosa, isolated from meat samples, in the agar disc diffusion assay, the efficacy of T. daenensis essential oil was negligible as it resulted in an inhibition zone of 19 mm only at 1 000 µg/mL.[47].

A polyphenol-rich fraction of T. daenensis methanol extract was evaluated for activity against Helicobacter pylori. Results showed that the effectiveness of this fraction was higher than the activity of the extract as a whole[48].

In an experimental study, T. daenensis essential oil and extracts of T. daenensis prepared with ethanol, ethanol 70% or water, were assessed for antifungal activity against Aspergillus flavus (AF), a food-borne fungus known for producing aflatoxins[49]. In a microplate assay the essential oil completely inhibited AF growth, and had an MIC of 375 mg/L, whereas the other extracts showed less activity. Additionally, AF spores treated with the essential oil (250 mg/L) did not germinate after 5 d of incubation, whereas those not treated with the oil showed 95% germination after only 8 h. The essential oil, at similar concentrations, was also effective against aflatoxin formation, which was assumed by the authors to be related to an inhibition of the ternary steps of aflatoxin biosynthesis, involving lipid peroxidation and oxygenation[49].

In another study, the activity of T. daenensis essential oil against C. albicans was moderate[50]. The inhibitory effect of T. daenensis on the mycelial growth of some pathogenic fungi was compared to other essential oils. This study reported a 92.74% ± 4.08% inhibition of mycelial growth at 20 µL T. daenensis essential oil/petri plate (an inhibition zone of 9 mm)[51].

3.2.2 Antioxidant and radical-scavenging activities

Numerous studies have been performed on the antioxidant and/or radical-scavenging properties of T. daenensis to underline the traditional uses of the plant against inflammatory diseases. For this purpose the essential oil and methanol extracts of T. daenensis were tested mainly in the 2,2-diphenyl-1-picryl hydrazyl (DPPH), nitric oxide (NO) and hydroxyl (OH) radical-scavenging as well as ferric-reducing antioxidant power (FRAP) and ferric thiocyanate (FTC) assays.

A methanolic extract was assessed by several of these methods. In the DPPH test, with a half maximal effective concentration (EC_{50}) of (194.24 ± 0.02) µg/mL, the extract showed approximately one tenth of the activity of quercetin and 25% of the one of butylated hydroxy toluene (BHT) as positive controls. NO radical-scavenging by the extract at a concentration of 200 µg/mL (74%) was higher than that of the essential oil (35%) and BHT (42%) at the same concentrations. OH radical-scavenging by the extract and the essential oil at 250 µg/mL (60.2% ± 0.3% and 54.7% ± 0.2%, respectively) was a little lower than those of gallic acid (70.8% ± 3.3%) and BHT (72.2% ± 3.7%)[25].

The radical-scavenging activity of a hydroethanolic extract in the DPPH assay was (1 906.5 ± 66.5) µmol Trolox/g extract. The FRAP assay resulted in the value of (962.0 ± 3.5) μmol/mL.[52]

In an isolated rat hepatocytes, the effects of a T. daenensis hydroalcoholic extract were evaluated via determination of malondialdehyde (MDA), as a marker for lipid peroxidation. MDA production was inhibited by up to 34% at 500 µg/mL of the extract, compared to the control. Serum glutamic oxaloacetic transaminase (SGOT) and low-density lipoprotein
(LDL) release from hepatic cells were reduced by 16% and 23%, respectively, as determined using colorimetry at concentrations of 500 µg/mL of the extract.[52]

In another study, the antioxidant and radical-scavenging activities of 90% methanol extracts of T. daenensis samples, collected during pre-flowering and full-flowering stages, were examined via DPPH and FRAP tests. EC₅₀ values were determined to be 8.46 and 8.23 µg/mL for DPPH radical-scavenging respectively. The FRAP test resulted in values of 24.23 and 26.45 µmol quercetin equivalents/g dry weight for the two stages of flowering, respectively.[39]. A significant DPPH-scavenging activity was shown for T. daenensis essential oil (EC₅₀ = (0.8 ± 0.06) µg/mL) in one study. In β-carotene bleaching the oil showed a dose-dependent effect between 60 µg/mL and 2.5 mg/mL.[21]. To assess the impact of heat treatment on the antioxidant activity of T. daenensis essential oil, the DPPH assay was employed. After heating of the oil to 120 ºC for 1, 2 or 3 h, the antioxidant and radical-scavenging activities were increased, whereas after heating to 180 ºC the antioxidant activity decreased.[40].

In a comparison of the antioxidant activities of several thyme species in the DPPH assay, the radical-scavenging of T. daenensis essential oil resulted in an EC₅₀ of (99.6 ± 0.5) µg/mL, and at approximately 250 µL/mL the inhibition in the β-carotene/linoleic acid bleaching assay was 88.4% ± 0.8%. The polar sub-fraction of the methanolic extract of this sample showed a higher DPPH-scavenging activity (EC₅₀ of (19.1 ± 0.1) µg/mL) than the other species and the essential oil. In β-carotene/linoleic acid bleaching the polar fraction exhibited the lowest activity. In contrast, the non-polar fraction of the methanolic extract was among the weakest samples in DPPH-scavenging (EC₅₀ of (267.5 ± 2.4) µg/mL), and at the most active in β-carotene/linoleic acid bleaching with an inhibition of 92.1% ± 1.1% at approximately 250 µg/mL.[27]. Thyme species endemic to Iran showed significant correlations between the total flavonoid content and DPPH radical, as well as 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) scavenging. The EC₅₀ values for a T. daenensis hydro alcoholic extract were 48.68, 210.90 and 23.68 µg/mL in the DPPH, ABTS and β-carotene bleaching assays, respectively.[7].

Potent superoxide anion and NO radical-scavenging activities were revealed for T. daenensis essential oil (EC₅₀ of 13.0 and 5.0 µg/mL, respectively). The ferric iron chelating activity of the essential oil was very weak.[53]. In the FRAP and DPPH assay, the antioxidant activity and radical-scavenging capacity of T. daenensis essential oil were higher than those of a commercial thyme oil (EC₅₀ DPPH = 5 µg/mL for T. daenensis and 17 µg/mL for the commercial sample; % of total antioxidant capacity via FRAP test was 17.81 ± 3.77 for T. daenensis and 0.23 ± 0.09 for the commercial sample).[39].

### 3.2.3 Tyrosinase inhibition

The tyrosinase-inhibitory activity of T. daenensis essential oil was evaluated spectrophotometrically, using the dopachrome method, in which quercetin served as positive control and L-dopa as a substrate. The half maximal inhibitory concentration (IC₅₀) for T. daenensis oil was determined to be 24 µg quercetin equivalent/g.[39].

### 3.2.4 Cytotoxic effects

Using the 3-(4,5-dimethyl-thiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assay, the cytotoxicity of the essential oil from T. daenensis and commercial thyme oil samples was evaluated for human normal lymphocytes as well as on HeLa cells. The IC₅₀ of T. daenensis essential oil was much higher than that of the commercial thyme oil (1 455 and 12.10 µg/mL, respectively) and thus, the cytotoxicity of T. daenensis essential oil on normal lymphocytes seems to be negligible. On the other hand, T. daenensis oil (IC₅₀ = 4.95 µg/mL) was as effective as the commercial thyme oil (IC₅₀ = 3.61 µg/mL) against HeLa cell line.[54]. Thus, the discrepancy in the activity of the two oils against normal human lymphocytes has to be confirmed.

### 3.2.5 Antifeedant and insecticidal properties

The secondary metabolites of several plants, including some essential oil components, are known as effective insecticides. Accordingly, the fumigant toxicity and repellent activity of T. daenensis essential oil against the first- and third-instar larvae and adults of Ephestia kuehniella (Mediterranean flour moth) and Plodia interpunctella (Indian flour moth) have been investigated. The oil showed fumigant toxicity against both insects in adult stage (lethal concentration (LC₅₀) = 0.191 and 0.27 µL/L_air respectively) while insects in larval stage were more resistant. Third instar larvae were significantly less sensitive than first instar larvae. The repellent activity of the oil against E. kuehniella (repellent concentration (RC₅₀) = 0.69 µL/L_air) was higher than against P. interpunctella (RC₅₀ = 6.88 µL/L_air)[22]. Additionally, a similar study showed the antifeedant activity of T. daenensis essential against the insect P. interpunctella Hubner with reference to the efficiency of conversion of ingested food to unit of body substance and feeding deterrence index.[55].

The fumigant toxicity of T. daenensis essential oil on Callosobruchus maculatus showed 92% mortality at a concentration of 18.52 µL/L_air. The LC₅₀ value of the oil was calculated to be 4.75 µL/L_air.[56]. Fumigant toxicity of T. daenensis oil was also assessed on Tribolium castaneum and T. confusum, with LC₅₀ values of 22.15 and 28.56 µL/L_air, respectively.[51].

Oviposition deterrence of T. daenensis oil against C. maculatus at the highest concentration of 0.24 µL/g mung bean seed (adult insects were first introduced to 5 g mung bean seeds) was 95.73%.[39].
3.2.6 Immunomodulatory effects

Many medicinal herbs are evaluated for their potential immunomodulatory effects. As a preliminary evaluation revealed that a *T. daenensis* aqueous extract stimulated the proliferation of human lymphocytes, the immunomodulatory effects of such an aqueous extract were investigated in more detail, namely on the activation of dendritic cells (DCs) and T lymphocyte cells. In the evaluation of general effects, the extract dose-dependently induced cell proliferation of activated-mouse spleen cells, resulting in proliferation indices of 1.98 ± 0.19 at 0.1 µg/mL to 1.44 ± 0.31 at 100 µg/mL and did not show signs of cytotoxicity on dendritic cells up to 200 µg/mL. In a study of how *T. daenensis* extract affected the expression of important co-stimulatory proteins in DCs, it was found that CD40 expression was enhanced whereas CD86 and major histocompatibility complex-II were not affected. The extract was not effective in the allogeneic mixed lymphocyte reaction or in influencing cytokine production of interferon-γ and interleukin-4.[58]

A hexane extract of *T. daenensis* showed strong inhibitory effects on phytohaemagglutinin-induced proliferation of peripheral blood lymphocytes. Thymol was determined to be the major constituent responsible for this effect, and as a pure compound reduced the proliferation by 62.8% at 50 µg/mL and by 89.8% at 200 µg/mL.[59]

3.2.7 Hypolipidemic properties

A hypolipidemic effect of *T. daenensis* essential oil was observed in a high-fat diet rat model after oral administration for three weeks. Rats received either normal diet, a high-fat diet with no additional intervention, a high-fat diet in combination with *T. daenensis* essential oil (200 mg/kg) or a high-fat diet in combination with Lovastatin (20 mg/kg). According to the results, *T. daenensis* essential oil and Lovastatin decreased the low-density lipoprotein level, as well as amounts of triglycerides in blood serum compared to those of the two other groups (*P < 0.05*).[60]

3.2.8 Toxicity and teratogenicity of thymol

Until now, no studies of toxicity or teratogenicity of *T. daenensis* have been performed. In most *T. daenensis* essential oil analyses, thymol was reported as the main constituent. Previous investigations revealed no considerable chronic cytotoxicity and teratogenicity for this compound.[61]

In some reports, stomatitis and cheilitis have been attributed to the use of thyme toothpastes, and some skin inflammations have been caused by hair shampoos.[62] Major symptoms in acute thymol toxicity have been described as dizziness, nausea, gastrointestinal complaints, headache and mental confusion.[63] The recommended daily oral dose for thyme is 1–4 g of dry herb.[62,63]

4 Conclusion and suggestions

*T. daenensis* is one of the Iranian endemic thyme species extensively administered by traditional practitioners. With thymol as main component of volatile constituents (almost 80% of total essential oil), the herb possesses antibacterial and antifungal activities. In spite of the variety of antimicrobial assessments, no antiviral evaluation has been performed on *T. daenensis* up to this point. Moreover, numerous antioxidant and radical-scavenging evaluations have been conducted on a wide variety of *T. daenensis* preparations. The findings of these studies may be considered as a basis for detailed investigation on anti-inflammatory properties, due to the presence of flavonoids and phenolic compounds. In contrast, only one animal study has been performed with *T. daenensis* until now. The promising effects of *T. daenensis* in a hyperlipidemic rat model should stimulate further investigations of its use in this manner. In general, there has been a lack of complementary in vivo studies and clinical trials investigating the pharmacological aspects of *T. daenensis*.

Most phytochemical analyses of *T. daenensis* have dealt with the composition of the essential oil and a few with the quantification of total phenolic compounds and flavonoids. Determining the bioactivity of other non-volatile isolates of *T. daenensis* would be important areas for further research.

As the herb is widely used by people in Iran, comprehensive studies on the chronic and acute toxicity and also teratogenicity of *T. daenensis* are recommended.

5 Conflicts of interest

Authors of this manuscript have no conflicts of interest.

REFERENCES

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