
# Chemical Composition and DPPH free Radical Scavenging Activity of *Tithonia diversifolia*

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**Abstract**
*Tithonia diversifolia* (Hemsl.) A. Gray (*Asteraceae*) is a plant widely distributed in tropical regions. Although used traditionally in several countries for sprains (Mexico), jaundice (China), and liver protection (Taiwan), in Vietnam it is primarily utilized as green manure due to its high phosphorus, calcium, and magnesium content. This study aimed to characterize the chemical constituents and evaluate the antioxidant potential, via DPPH free radical scavenging, of ethanol leaf extracts of *T. diversifolia* collected in Buon Ma Thuot, Dak Lak Province, Vietnam. Chemical profiling was performed using liquid chromatography–mass spectrometry (LC–MS). Antioxidant activity was assessed by the DPPH assay, with ascorbic acid serving as a positive control. LC–MS analysis identified sesquiterpenes and phenolic compounds as the major chemical classes in the ethanol extract. The extract exhibited moderate DPPH scavenging activity, with an $IC_{50}$ value of 38.85 mg/mL, compared to 34.99 mg/mL for ascorbic acid. The ethanol extract of *T. diversifolia* leaves contains bioactive sesquiterpenes and phenolics with moderate free radical scavenging capacity, supporting its potential as a natural antioxidant source.

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## Introduction

*Tithonia diversifolia* (Hemsl.) A. Gray (*Asteraceae*), commonly known as Mexican sunflower, is a perennial shrub (often considered a weed) native to Central America but now naturalized across many tropical regions, including Nigeria. In Nigeria, the species is frequently found along roadsides, in cultivated fields, and in abandoned lands [1]. It typically flowers in October, producing approximately 80,000–160,000 seeds annually, with germination rates ranging from 18% to 56% at 25 °C. Seed dispersal is facilitated by human activity, livestock movement, and water flow. The plant generally reaches 1.2–3 m in height. Leaves are alternate, lobed (though upper leaves may be unlobed), with cuneate or attenuate bases, acuminate apices, and serrated margins, measuring 5–17 × 3.5–12 cm. The abaxial surface is densely pubescent, and venation is palmately patterned. Inflorescences are solitary on peduncles 6–13 cm long; ray florets are bright yellow, measuring 3–6 cm × 5–18 mm. Diagnostic morphological features of *T. diversifolia* include both glandular and non-glandular trichomes (capitate and non-capitate) on the leaf surface and veins, as well as secretory canals located in close proximity to the vascular bundles [2].

Traditionally, all parts of *Tithonia diversifolia*, particularly the leaves, have been widely used by indigenous communities to treat a variety of ailments. These include topical applications for wound healing, musculoskeletal disorders, abscesses, dermatological conditions, and stomach pain, as well as systemic treatments for diabetes, malaria, fever, hepatitis, and various infectious diseases [3]. Despite its invasive nature, the species has been employed as an organic fertilizer to improve yields of vegetable and maize crops in Nigeria and Kenya [4]. Historically, *T. diversifolia* has been sought after by traditional healers for its medicinal value, with several reported phytopharmacological and ethnopharmacological activities [5]. For instance, administration of leaf extract at 400 mg/kg/day significantly reduced parasitemia in mice infected with *Trypanosoma brucei*.

The essential oil of *T. diversifolia* prolonged protection time against mosquito species such as *Anopheles gambiae*, *Aedes aegypti*, and *Culex quinquefasciatus*, while methanol leaf extract exhibited toxicity toward *A. gambiae* larvae [6]. In another study, the antibacterial activities of ethyl acetate and methanol leaf extracts were evaluated against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*. Additionally, soap formulated with aqueous leaf extract (0.0–15% w/w) significantly inhibited the growth of *E. coli* and *Candida albicans* [7].

In Vietnam, *Tithonia diversifolia* (Hemsl.) A. Gray (*Asteraceae*) was first introduced by the French for cultivation on plantations in Lam Dong Province. It was primarily grown as green manure for coffee and rubber plantations due to its high phosphorus, calcium, and magnesium content in the stems, which makes it a valuable organic fertilizer. The leaves have also been used in traditional folk medicine to treat scabies. Due to its ability to propagate easily via seeds and stem cuttings, as well as its efficient seed dispersal, *T. diversifolia* has naturalized across the Northern region, several Southern provinces, and the Central Highlands, including Buon Ma Thuot City. In Vietnam, previous research has focused on the isolation and purification of tagitinin C from *T. diversifolia*, achieving pilot-scale production of 100 g meeting the national quality standard. The safety profile and antiplasmodial activity of tagitinin C were evaluated both in vitro and in vivo under the framework of the National Key Science and Technology Program for the Development of the Pharmaceutical Industry to 2020, led by the Ministry of Industry and Trade. Furthermore, a method for preparing bioactive extracts with anticancer potential from *T. diversifolia* leaves was developed [8].

However, in Vietnam, the plant has remained primarily used as organic fertilizer, and studies on its chemical composition and bioactivities remain limited. Therefore, this study was conducted to determine the chemical constituents and evaluate the bioactivity of *T. diversifolia* ethanol leaf extract, aiming to provide systematic and comprehensive data that could serve as a scientific basis for its effective utilization.

## Materials and Methods

### Research materials

Leaves of *T. diversifolia* were collected from Tan Hoa Ward, Buon Ma Thuot City, Dak Lak Province, in October 2022.

### Preparation of Ethanol Extract

Leaves were thoroughly washed, air-dried, and cut into small pieces. The dried plant material was macerated in a sealed glass container with 99.5% ethanol at room temperature for 10 days to allow solvent penetration into the plant cell walls and dissolution of phytochemicals. The mixture was filtered through filter paper, and the filtrate was concentrated to dryness under reduced pressure to obtain the ethanol extract of *T. diversifolia* leaves.

**Fig 1:** Dried leaves of *T. diversifolia* after sun-drying

### Chemical Composition Determination Method

The chemical composition of the extract was analyzed using Liquid Chromatography–Mass Spectrometry (LC–MS). The analysis was conducted at the Institute of Biotechnology and Environment, Tay Nguyen University.

### DPPH Free Radical Scavenging Activity Assay

The antioxidant activity of *Tithonia diversifolia* was evaluated at the Institute of Biotechnology and Environment, Tay Nguyen University. The assay was performed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method. Test samples were dissolved in methanol, and ascorbic acid was used as a positive control.

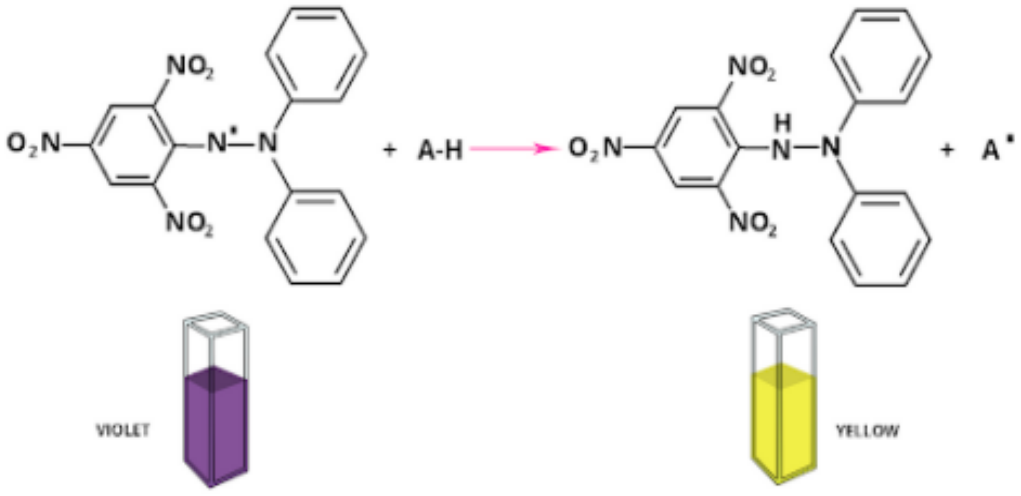



**Fig 2:** Color change of the DPPH solution before and after the reaction.

The DPPH free radical scavenging capacity was calculated using the following equation:

$$\%IC = \frac{OD_{Control} - OD_{Sample}}{OD_{Control} - OD_{Blank}} \times 100\%$$

In which:
$OD_{Control}$: absorbance of the control sample (without extract)OD thử: độ hấp thu của mẫu
$OD_{Sample}$: absorbance of the test sample
$OD_{Blank}$: absorbance of the blank sample (methanol)
The $IC_{50}$ value of both the test sample and the positive control was determined based on the linear regression equation between concentration and % radical scavenging activity, according to the formula:

$$IC_{50} = \frac{50 - b}{a}$$

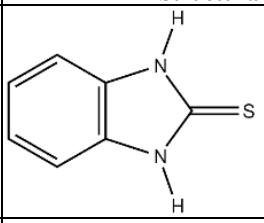
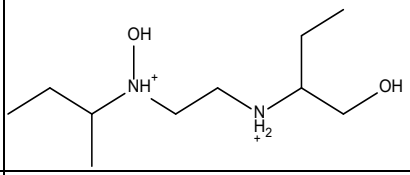
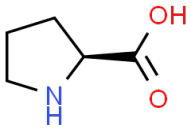
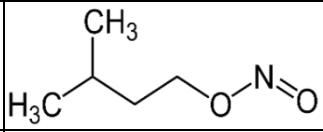
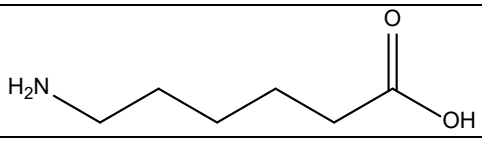
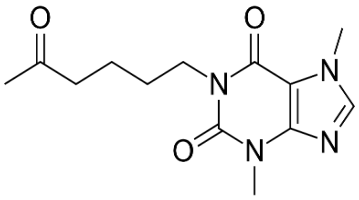
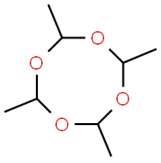
In which:
$IC_{50}$: concentration of the sample required to scavenge 50% of DPPH free radicals
a,b: slope and intercept, respectively, of the linear regression equation between concentration and % scavenging activity.

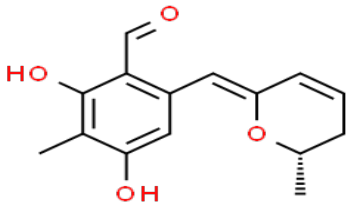
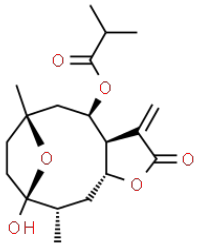
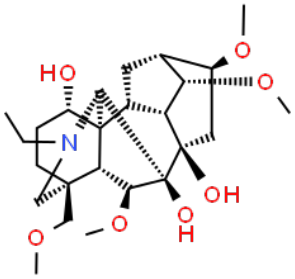
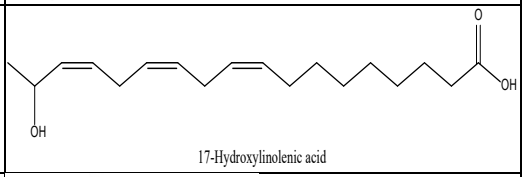
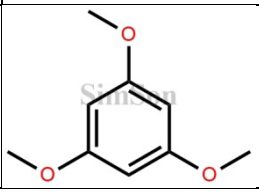
## Results and Discussion

### Chemical composition of *Tithonia diversifolia*

The chemical composition of the ethanol extract of *Tithonia diversifolia* leaves was analyzed using LC–MS (Liquid Chromatography–Mass Spectrometry), and the results are presented in Table 1.

**Table 1:** Chemical constituents of the ethanol extract of *Tithonia diversifolia* leaves

| No. | Compound | Molecular Formula | Structural Formula |
|---|---|---|---|
| 1 | Mercaptobenzimidazole | $C_7H_6N_2S$ |  |
| 2 | 1-hydroxybutan-2-yl-[2-(1-hydroxybutan-2-ylazaniumyl)ethyl]azanium | $C_{10}H_{26}N_2O_2$ |  |
| 3 | Allylglycine | $C_5H_9NO_2$ |  |
| 4 | Amylnitrite | $C_5H_{11}NO_2$ |  |
| 5 | Aminocaproic acid | $C_6H_{13}NO_2$ |  |
| 6 | Pentoxifylline | $C_{13}H_{18}N_4O_3$ |  |
| 7 | Metaldehyde | $C_8H_{16}O_4$ |  |

| | | | |
|---|---|---|---|
| 8 | Cyperin | $C_{15}H_{16}O_4$ |  |
| 9 | Tirotundin | $C_{19}H_{28}O_6$ |  |
| 10 | Delsoline | $C_{25}H_{41}NO_7$ |  |
| 11 | 17-Hydroxylinolenic acid | $C_{18}H_{30}O_3$ |   |
| 12 | Phloroglucinol trimethyl ether | $C_9H_{12}O_3$ |  |

Remarks: From the table above, we observed that the major constituents in the ethanol extract of *Tithonia diversifolia* leaves are consistent with previous reports, mainly sesquiterpenes and phenolic compounds. In addition, several other compounds such as bicyclic compounds, amides, and alkaloids were also detected [9, 10].

**DPPH free radical scavenging activity of the ethanol extract**

The ethanol extract of *Tithonia diversifolia* leaves was tested for DPPH free radical scavenging activity, using ascorbic acid as the positive control. The results are presented in Table 2.

**Table 2:** Antioxidant activity (DPPH radical scavenging)

| Extract concentration (mg/ml) | % Inhibition | $IC_{50}$ value (mg/ml) | Ascorbic acid $IC_{50}$ (mg/ml) |
|---|---|---|---|
| 10 | 32.19 | 38.85 | 34.99 |
| 20 | 37.92 | | |
| 40 | 50.09 | | |
| 60 | 63.15 | | |
| 80 | 76.18 | | |
| 100 | 90.16 | | |

Remarks: The results indicate that the ethanol extract exhibited strong DPPH radical scavenging activity, with an $IC_{50}$ value of 38.85 mg/ml, which is comparable to the positive control, ascorbic acid ($IC_{50}$ = 34.99 mg/ml). This finding is consistent with previous reports [10, 11, 12].

**Conclusion**

In this study, we investigated the chemical constituents of the ethanol extract from *Tithonia diversifolia* leaves collected in Dak Lak province, Vietnam. The isolation and identification of compounds were performed using Liquid Chromatography–Mass Spectrometry (LC–MS) analysis. The main classes of compounds identified were sesquiterpenes and phenolic compounds.

The antioxidant activity assay using the DPPH free radical scavenging method revealed that the ethanol extract possessed a notable antioxidant capacity, as indicated by an $IC_{50}$ value of 38.85 mg/ml, comparable to that of the positive control, ascorbic acid ($IC_{50}$ = 34.99 mg/ml). These promising results, together with further in-depth studies, may open up

the potential for exploiting *T. diversifolia* as a source of functional foods and pharmaceuticals, contributing to the effective utilization of this plant in Vietnam.

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