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Article

Tridax procumbens (Asteraceae): A Morphoanatomical Characterization of Its Inflorescences, Chemical and Toxic Potential Investigation of its Essential Oil

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RESUMO

Tridax procumbens L. (Asteraceae) é uma espécie nativa não endêmica com ampla distribuição no Brasil e com predomínio em áreas antropizadas. Na medicina popular, essa planta é utilizada no tratamento de diarréia, redução de secreções brônquicas, como antiviral, cicatrizante, diabetes, anti-séptica, dentre outros usos. Nesse contexto, este estudo realizou a caracterização morfoanatômica das inflorescências de *T. procumbens*, a análise fitoquímica do óleo volátil extraído dessas estruturas, bem como a avaliação da sua toxicidade por meio do ensaio com *Artemia salina* e atividade antimicrobiana frente a *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumoniae* e *Pseudomonas aeruginosa*. Os resultados demonstraram que as inflorescências terminais possuem cabeça tipo solitária, irradiada por brácteas involucrais e recoberta por tricomas, flores de raia ligadas e disco tubular de ambos os sexos. O óleo volátil apresentou 21 compostos químicos - terpenos com a presença de monoterpenos e sesquiterpenos usados como mistura de compostos em diversas aplicações como cosméticos, alimentos e medicamentos. O óleo não apresentou toxicidade para *Artemia salina* e apresentou atividade antimicrobiana moderada contra *Staphylococcus aureus* com CMI de 250 $\mu g.mL^{-1}$. Nosso estudo contribui para a identificação da morfoanatomia floral e a identificação da composição química do óleo volátil que podem ser relevantes para o controle de qualidade na produção de medicamentos com matéria-prima da inflorescência de *T. procumbens*. Além disso, o conhecimento farmacognóstico das características químicas do óleo volátil das inflorescências de *T. procumbens* agregam informações importantes relacionadas ao aroma, condimentos e antioxidantes, bem como às suas atividades biológicas.

Palavras-chave: Fitoquímica; Plantas medicinais; *Artemia salina*; Bactérias patogênicas; Produtos naturais.

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ABSTRACT

Tridax procumbens L. (Asteraceae) is a native non-endemic species with ample distribution in Brazil, predominantly in anthropic areas. In folk medicine, this plant is used in the treatment of diarrhea, reduction of bronchial secretions, antiviral, wound healing, diabetes, antiseptic, among other uses. In this context, this study carried out the morphoanatomical characterization of *T. procumbens* inflorescences, the phytochemical analysis of the volatile oil extracted from these structures, as well as the evaluation of its toxicity using the *Artemia salina* assay and antimicrobial activity against *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. The results showed that the terminal inflorescences have a solitary head, irradiated by involucral bracts and covered by trichomes, connected ray flowers and tubular disc of both sexes. The volatile oil had 21 chemical compounds - terpenes with the presence of monoterpenes and sesquiterpenes used as a mixture of compounds in various applications such as cosmetics, food and medicine. The oil did not show toxicity to *A. salina* and showed moderate antimicrobial activity against *Staphylococcus aureus* with MIC of 250 μg.mL^{-1}. Our study contributes to the identification of the floral morphoanatomy and the identification of the chemical composition of the volatile oil, which may be relevant for quality control in the production of medicines with raw material from the inflorescence of *T. procumbens*. Furthermore, the pharmacognostic knowledge of the chemical characteristics of volatile oil from *T. procumbens* inflorescences add important information related to aroma, spices and antioxidants, as well as their biological activities.
Keywords: phytochemistry; medicinal plants; *artemia salina*; pathogenic bacteria; natural products.

Introduction

Medicinal plants represent a significant source of chemical compounds with therapeutic potential and are widely used in phytotherapy. Their popular use is a relevant alternative, both due to the efficacy traditionally recognized by communities and the accessibility they offer to economically disadvantaged or geographically isolated populations (Vila Verde et al. 2003; Stefanello et al. 2018). In this context, ethnodirected approaches are essential to guide phytochemical studies and evaluate biological activities with therapeutic potential, contributing to the discovery and development of new drugs applicable to both traditional and modern medicine (De Albuquerque & Hanazaki 2006).Thus, an ethno directed approach becomes essential in directing phytochemical studies and determining biological activities with an emphasis on therapeutic potential. In this context, this approach contributes to the discovery and development of new drugs with applications in phytotherapy and medicinal chemistry as prototypes for new medicines (De Albuquerque & Hanazaki 2006).

Brazilian biodiversity provides a promising landscape for research in the pharmaceutical field, and the Asteraceae family (Bercht. & J.Presl) stands out as the most diverse among angiosperms, comprising approximately 30,000 species distributed in 1,700 genera. In Brazil, 2,092 species are registered across 279 genera, of which 1,345 species and 67 genera are endemic, occurring in all phytogeographic domains and types of vegetation (Hind & Andrade 2024; Flora do Brasil 2024). One of the most notable morphological characteristics of this family is the presence of a capitulum-type inflorescence, usually small, with flowers surrounded by bracts, a syngenesious androecium, and cypsela-type fruits with pappus (Mondin 2015).

Among the various genera of the family, the genus *Tridax* was selected for this study due to its wide distribution in the Americas and its relevance in folk medicine. The genus comprises 26 species and two varieties known in South and Central America and Mexico, characterized by bilabiate corollas in the ray flowers (Powell 1965). In Brazil, only one species occurs—*Tridax procumbens* L.—classified as native but not endemic. It is an annual or biennial herbaceous plant, widely distributed throughout the country, especially in disturbed environments such as pastures, sidewalks, and cultivated areas, where it is often considered a weed due to its aggressive growth (Mondin 2015; Powell 1965).

The choice of *T. procumbens* for this research is justified by its wide distribution, high adaptability to disturbed environments, and the numerous biological activities already reported in the literature, including antimicrobial, anti-inflammatory, wound healing, and antidiabetic properties (Taddei & Rosas-Romero 2000; Manjamalai et al. 2012). However, there is a significant gap: previous studies rarely specify which plant parts were used, and there is no consistent report on the medicinal use of the inflorescence. It is presumed that the leaves, stems, and roots are commonly employed in preparations, while the inflorescence remains neglected.

This lack of information highlights the problem highlighted in this study, which aims to fill this scientific gap by specifically investigating the plant's inflorescences. Considering the morphological variability of Asteraceae species in different ecological conditions and the absence of data on the medicinal use of this structure, it is relevant to provide a more precise description of the morphology of the *T. procumbens* inflorescence. This not only contributes to taxonomic confirmation among different regions but also paves the way for identifying potentially pharmacologically active components that have not yet been described.

Furthermore, essential oils extracted from Asteraceae inflorescences have shown promising antimicrobial activity, especially when they contain monoterpenes such as 1,8-cineole (Bizzo 2009). However, factors such as collection time, climate, and soil type directly influence the chemical composition of these oils (Taddei & Rosas-Romero 2000; De Albuquerque & Hanazaki 2006). Therefore, specifically studying the essential oil of *T. procumbens* inflorescences may bring relevant contributions to both botanical knowledge and therapeutic applications.

Thus, the objective of this study is to analyze the floral morphoanatomy of *Tridax procumbens* L. (Asteraceae) inflorescences, perform chemical characterization of the volatile oil obtained from this structure, and investigate its toxicity and antimicrobial activity. These analyses are relevant to establish pharmacognostic parameters for quality control of the raw material, considering that *T. procumbens* inflorescences, widely used in Brazilian folk medicine, are cultivated in the savannah region. This approach will not only expand the morphoanatomical understanding of the species but also assess its pharmacological potential.

Methodology

Vegetable material

We collected the samples of the inflorescences of *Tridax procumbens* L. (Asteraceae) at the Central Park Senador Onofre Quinan in Anápolis, at the geographical coordinates 16° 20' 19" S 48° 57' 53" O, in the early hours of the morning, in March 2019. An exsiccate of the plant species were deposited under the number 12438 by Professor Mirley Luciene Santos, the curator of the Herbarium of the State University of Goiás.

After, the inflorescences were dried in an oven with circulation and air renewal Solab SL-102, at 40°C for 48 hours. Then they were sprayed in a knife mill of the brand Marconi, model MA580. The size of the granulometric sieves was 0.85mm. The powder was stored in a cool and dry place, protected from light.

Morphoanatomical analysis of inflorescence

The morphoanatomical characterization of *Tridax procumbens* inflorescences was carried out through the observation of fresh samples using a Leica stereoscopic magnifier equipped with an attached camera. For the microscopic description, transverse and paradermal sections of the fresh inflorescences were prepared. These sections were cleared using 6% sodium hypochlorite to enhance contrast for anatomical visualization, followed by rinsing in distilled water.

For histochemical analysis, the sections were stained with methylene blue to highlight general anatomical features and with Sudan III to detect lipid-containing structures and oleaginous compounds. These stains facilitated the identification of tissues and cellular components associated with essential oil storage and secretion.

Microscopic analyses were conducted using a Leica light microscope with up to 1,000× magnification. Measurements of peduncle length and flower distribution were performed with the aid of graph paper, following a method adapted from Bianco et al. (2004). Morphometric data were obtained from 10 fully open inflorescences from each of 30 different individuals, totaling 300 inflorescences, in accordance with Capucho (2008).

Characterization of volatile oil

We used the hydrodistillation method, with a Clevenger type apparatus, to carry out the extraction of volatile oil from the inflorescence of *T. procumbens*. For that, 100 g of the dried material powder was used in 1,000 mL of distilled water and kept for three hours. We dried the extracted volatile oil over anhydrous sodium sulfate (Na_2SO_4) and repeated the process three times. We kept the oil in an amber bottle under refrigeration (- 20 ºC) according to Barros et al. 2018.

The volatile oil obtained was subjected to gas chromatographic analysis, coupled to mass spectrometry (CG/MS), in a SHIMADZU GCMS-QP2010 Ultra apparatus. We prepared the sample used in the chromatography in 1:10. We dissolved the oil in hexane grade HPLC, with the injection of 1µl in the RTX-5MS capillary column of 30 cm in length and 0.25 mm of internal diameter, using the helium gas for the entrainment with the flow of 1 mL/min, with an initial temperature of 60 °C for 2 min. Then the temperature was increased until it reached 240 °C at a rate of 3 °C/min^{-1}. Then it was increased until reaching 280 ºC, with a rate of 10 ºC/min^{-1} maintaining this temperature for 10 minutes and ionization energy of 70 eV. The injector temperature was 250 °C and operated in Split Less: 20 mode. The compounds were identified using a computerized database, using the digital mass data library NIST11/2011/EPA/NIH and by comparison with their retention indices and authentic mass spectra reported in the literature for the most common components of volatile oils (Adams 2007).

As a standard, we performed a co-injection of a mixture of hydrocarbon C8-C32 (Sigma-Aldrich) and used the arithmetic index calculation according to the equation of Van Den Dool and Kratz (1963) described below:

IR= 100.N[(tx – tn)/(tn – tn-1)]+100.Cn – 1

Where:

N = Cn – Cn-1

Cn = number of carbons of the n-alkane that elutes after the analyzed substance

Cn-1 = number of carbons of the n-alkane that elutes before the analyzed substance

tx = retention time of the analyzed substance

tn = retention time of the n-alkane that elutes after the analyzed substance

tn-1 = retention time of the n-alkane that elutes before the analyzed substance

Toxicity test

The test performed was adapted according to the methodology of Choudhary et al. 2001. Briefly, 250 mg of *Artemia salina* L. cysts were incubated for 40 hours in artificial seawater medium with 3.6 % NaCl (w/v) supplemented with 6 mg.L^{-1} of yeast extract, under constant oxygenation, at room temperature with natural light.

After hatching, nauplii were attracted with artificial lighting and transferred to a Petri dish with fresh seawater medium. Then, 10 nauplii were transferred to individual wells of a 96-well microplate. We performed the toxicity test at concentrations of 2,000, 1,000, 500, 250, 125, and 62.5 µg.mL^{-1} of the volatile oils dissolved in dimethyl sulfoxide (DMSO) 5% and seawater medium with 0.02% Tween 80®. Controls were included as viability controls for *A. salina*, technique controls with potassium dichromate ($K_2Cr_2O_7$), DMSO 5%, tween 0.02%, and uninoculated controls.

After incubation for 24 hours at room temperature, we observed the mortality of nauplii of *A. salina* in each well and calculated the 50 % lethal concentration (LC_{50}). We obtained the correlation between the percentage of individuals killed and the concentration of the test substance using the Probit regression method with the StatSoftStatistica 10 program (Analyst Soft/Probit) (Svensson et al. 2005). All experiments were carried out in independent triplicates:

Antimicrobial activity

The determination of the antimicrobial activity was performed by the broth microdilution method, with a protocol standardized by the Clinical and Laboratory Standard Institute, in an antimicrobial susceptibility test by diluting the antimicrobial agents in broth (CLSI, 2016), using the following microorganisms: *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25312, *Klebsiella pneumoniae* ATCC 700603, and *Pseudomonas aeruginosa* ATCC 27853.

We performed the determination of the minimum inhibitory concentration (MIC) in sterile 96-well microdilution plates with U-bottom. Initially, it was solubilized the pure volatile oil in 5% DMSO. Later, it was solubilized it in Mueller Hinton broth with 0.02% tween 80 at the concentrations of 2,000, 1,000, 500, 250, and 125 $\mu g.mL^{-1}$. We used the chloramphenicol (Inlab) as technique control in concentrations of 64, 32, 16, 8, and 4 $\mu g.mL^{-1}$. MIC was defined as the lowest concentration of the compound able to completely inhibit visible microbial growth. We carried out the experiments in independent triplicates (CLSI 2016):

Results and Discussion

Morphoanatomical analysis of inflorescences

The specimens of *Tridax procumbens* analyzed in this study were collected from ruderal environments exposed to full sunlight (Figure 1). The inflorescences are of the capitulum (head) type, typical of the Asteraceae family, composed of a common receptacle bearing two types of flowers: ray flowers with ligulate, gamopetalous corollas, and tubular disc flowers with pentamerous, gamopetalous corollas, exhibiting an elongated tube and short lobes. Both flower types are hermaphroditic and present trichome-covered corolla tubes and bifid, feathery stigmas.

In the morphological quantification of 300 inflorescences from 30 individuals, we observed peduncle lengths ranging from 7 to 17 cm (Figure 2A). Each capitulum had five to six ray flowers and a variation of 48 to 104 disc flowers, totaling 2,168 tubular disc flowers—an average of 67 disc flowers per capitulum (Figure 2B). These results reveal considerable variation in floral number within the species.

Microscopic observations showed the presence of paleae (chaffy bracts) between the disc flowers and involucral bracts with prominent trichomes surrounding the floral base and peduncle (Figure 3C). The ray and disc flowers also displayed distinct morphological traits: the ligulate ray flowers had expanded limbs, while the disc flowers were cylindrical with fused lobes (Figure 3A, B). Trichomes were abundant on the outer surfaces of both floral types (Figure 3D), and the distribution and shape of these structures were consistent with descriptions for the family (Roque & Bautista, 2008; Mondin, 2015), reinforcing the identification and typicality of the species.

Therefore, the data observed in this study corroborate previous reports in the literature on *T. procumbens* and other Asteraceae species, while also contributing new quantitative information on floral number and peduncle length in inflorescences from native populations.

Usually, the flowers of the Asteraceae family have five synantheric stamens, with the stylets surrounding the corolla of the flower. The gynoecium has an unicarpelar inferior ovary where a simple stylus with bifid stigmas inserted, and its fruits are of the cypsela type: syncratic, unilocular, and monospermic, originating from the inferior ovary (Mondin 2015; Gandara et al. 2016; Zugaib & Amorim 2015).

Microscopic analyses of the *Tridax procumbens* inflorescences revealed several important anatomical features. The floral structures included synanterous stamens, characterized by filaments fused to form a tube and anthers joined into a ring (Figure 3F). The gynoecium consisted of an inferior ovary and a bifid style with feathery stigmatic branches at the upper extremity (Figure 3F, G), typical of the Asteraceae family.

Anatomical sections (cross and paradermal) showed that the inflorescence is composed of epidermal, parenchymatic, and vascular tissues, with evident protective and secretory adaptations. Among these, we identified two types of trichomes: tector trichomes (non-glandular), which were mostly multicellular and uniseriate, and glandular trichomes with a multicellular head and short stalk (Figure 4A, B, D, E). These trichomes were abundant on the surface of the floral organs and peduncle. Glandular trichomes were shown to contain lipid substances and volatile oils, as evidenced by Steinmetz's histochemical reaction. These compounds were observed exclusively in the glandular trichomes. In addition, the epidermis presented anomocytic stomata, as shown in Figure 4C.

These secretory and epidermal characteristics suggest a protective and potentially allelopathic role, consistent with observations in other Asteraceae species. Similar findings were described by Ramos et al. (2022), who conducted a pharmacobotanical characterization of *T. procumbens*, identifying comparable trichome types and using histochemical tests with Sudan III to detect lipophilic substances. Their study, however, focused on roots, stems, and leaves. Our findings extend anatomical descriptions to reproductive organs, reinforcing the structural complexity and functional diversity of this species.

Regarding pollen morphology, Figure 3H illustrates oblate-spheroidal pollen grains, predominantly small to medium in size, with echinate ornamentation—a profile in agreement with Galvão et al. (2009), who emphasized the pollen variability within the Asteraceae family. These features confirm the identity of *T. procumbens* and align with the morphoanatomical patterns expected for this taxonomic group.

Figure 1. Growth environments of *T. procumbens*. A – Moist soil, B – stony soil, and C – the sidewalk. The species demonstrates adaptability to various substrates. Source: Prepared by the author (2025).

Figure 2. Variation in peduncle length of *Tridax procumbens* capitulum (head inflorescence), arranged in ascending order over a graph paper background to facilitate comparison. Source: Prepared by the author (2025).

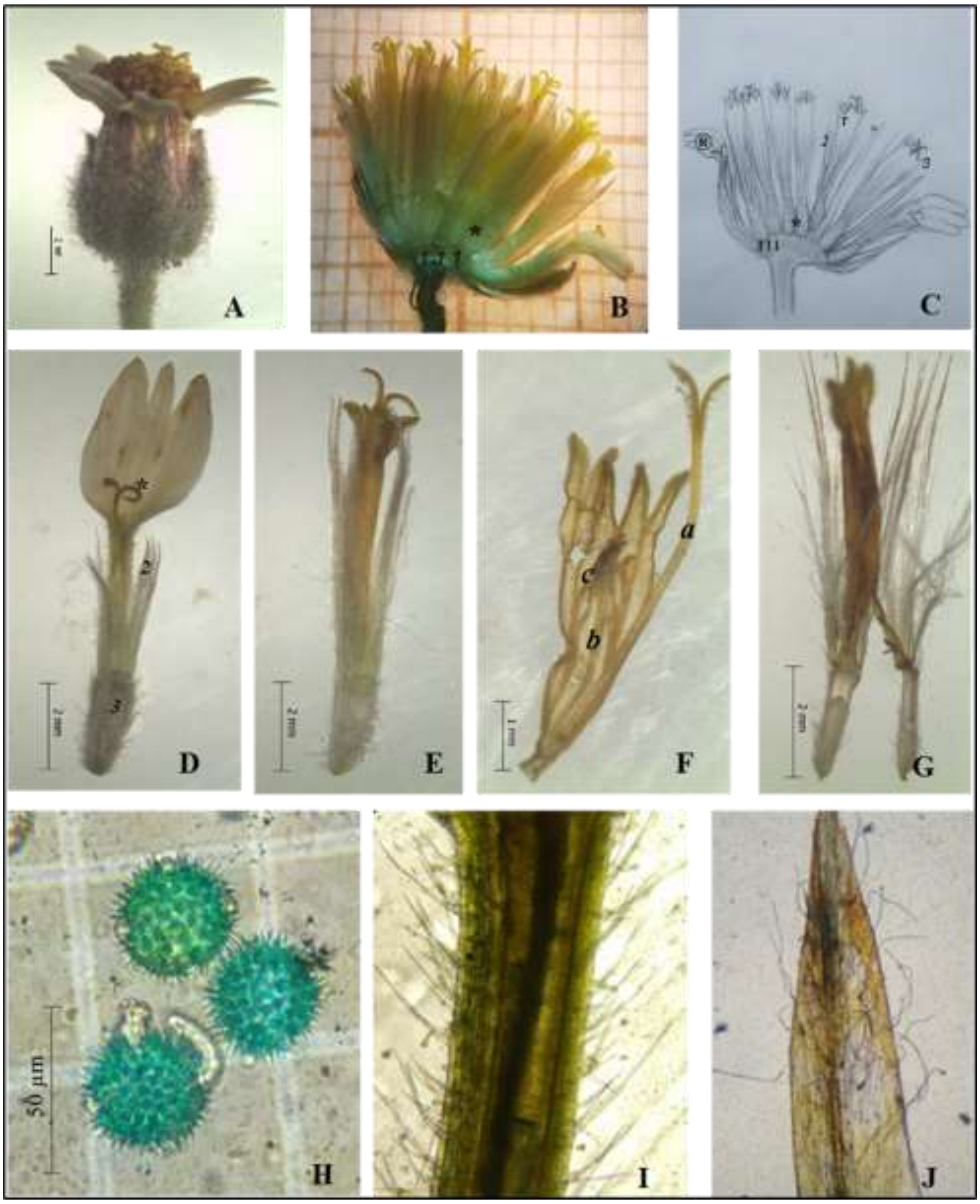

Figure 3. Morphology and anatomy of inflorescence and flowers of T. procumbens. Source: Prepared by the author (2025)

- A - solitary radiated head;
- B - cross-section of the radiated head, 111 showing the receptacle and *ovary;
- C - diagram of the head: 111 receptacles, * ovary, ® river flower, T tubular flower, > pale, 2 papules, 3 lacinia,
- D - ligulated ray flower, * pistil, 2 papules, 3 ovaries;
- E - tubular disk flower;
- F - open corolla showing synanterous stamens;
- G - three-layer ovary and ovum;
- H - pollen grain stained with methylene blue with 1,000 X magnification;
- I - three-layer tubular corolla;
- J - pale (interfloral bract) stained with Sudan III showing lipophilic substances.

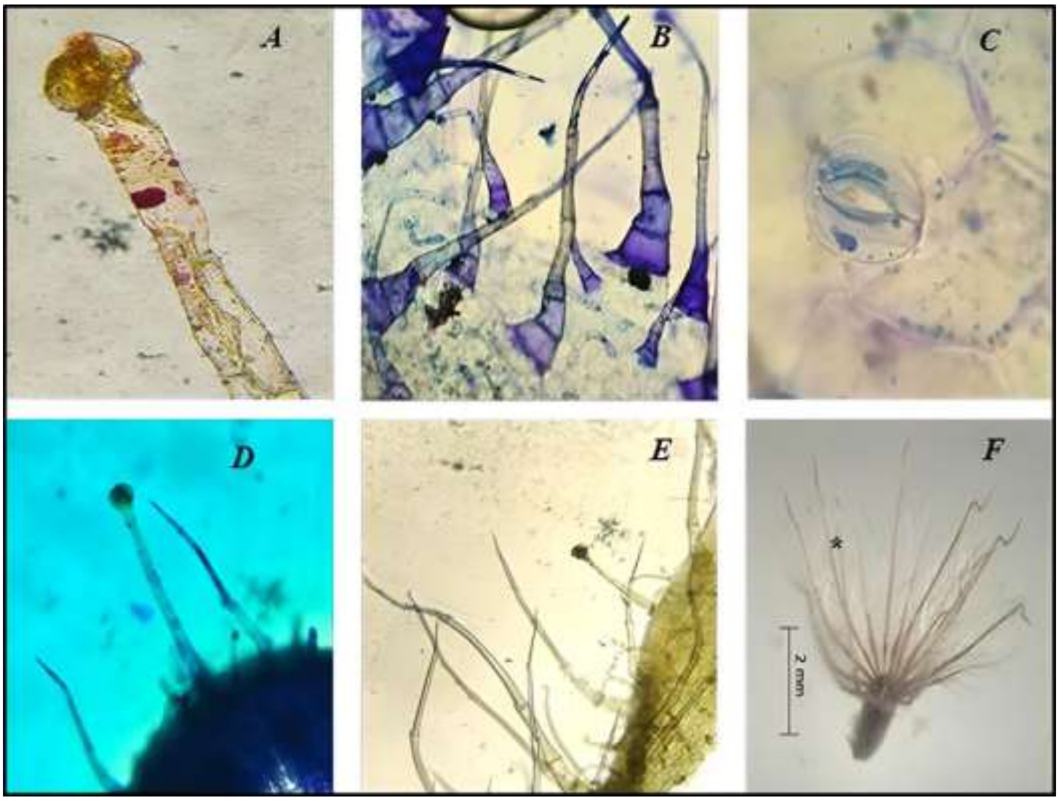

Figure 4: Floral anatomy and morphology of the fruit of *T. procumbens*. Source: Metcalfe & Chalk (1950)

- A - glandular trichomes with lipid substances and volatile oil;
- B - paradmal cut of the involucral bract showing the trichomes, tectors, and glands;
- C - paradmal cut of the involucral bract evidenced by a stoma;
- D - cross-section of the peduncle evidenced with methylene blue;
- D - paradermic cut of the peduncle showing the trichomes with Sudan III dye;
- F - cypsela type fruit * double feathery, feathery pappus.

Chemical characterization of volatile oil

The volatile oil from the dry inflorescences of *T. procumbens* was slightly yellow liquid, with low viscosity, and with characteristic odour. We identified 21 compounds in the volatile oil sample chemical composition, mostly terpenes in the form of monoterpenes and sesquiterpenes: 2,4,6-trimethyldecane (37.83%), α-ionone (10.42%), 2-hydroxymethyl-hexan-1-ol (9.30%), undecanal (6.18%), and tetrahydro geraniol (6.16%). Fatty acids, such as oleic acid and stearic acid, have also been identified with applications in the food and pharmaceutical fields due to their antioxidant properties explored in the prevention of degenerative diseases (Oliveira 2013). We organized the chemical components in order of elution according to the chromatogram of the GC/MS analysis (Table 1).

The dominant compounds belong to the hydrocarbon, acetone, and ester groups. Other researches have shown volatile compositions with monoterpenes and sesquiterpenes predominance in other Asteraceae species (Fabiane et al. 2008; Manjamalai et al. 2012) and report that monoterpenes and sesquiterpenes predominate in the composition of the volatile oil of *T. procumbens* leaves.

Table 1: Chemical constituents of the volatile oil in the inflorescences of T. procumbens.

T	RI	Compound name	MM	RA%	Formula
13.360	1121	2,4,6-Trimethyldecane	184	37.83	$C_{13}H_{28}$
13.990	1134	tetrahydrogeraniol	186	6.16	$C_{11}H_{22}O_2$
14.138	1138	2-hydroxymethyl-hexan-1-ol	136	9.30	$C_7H_{16}O_2$
20.005	1263	undecylenic alcohol	156	4.25	$C_{10}H_{20}O$
21.275	1296	(R) - (+) - citronellic acid	170	2.90	$C_{10}H_{18}O_2$
24.414	1369	Undecanal	170	6.18	$C_{11}H_{22}O$
25.230	1388	trans-β-damascenone	190	0.57	$C_{13}H_{18}O$
25.579	1071	formic acid	136	1.47	$C_8H_8O_2$
25.790	1428	dictamnol (none)	178	3.90	$C_{12}H_{18}O$
27.031	1431	α-ionone	192	10.42	$C_{13}H_{20}O$
35.205	1641	bisaboladienol	208	2.55	$C_{15}H_{26}O$
34.135	1608	β-atlantol	220	1.69	$C_{15}H_{24}O$
29.795	1499	α-himalachene	204	0.73	$C_{15}H_{24}$
36.586	1678	cyclotetradecane	196	1.29	$C_{14}H_{28}$
37. 434	1700	n-heptadecane	240	0.46	$C_{17}H_{36}$
38.430	1730	methyl-isonanoate	254	1.51	$C_{16}H_{30}O_2$
45.509	1849	2-heptadecanone	254	2.10	$C_{17}H_{34}O$
48.420	2034	farnesyl butyrate	292	0.60	$C_{19}H_{32}O_2$
52.543	2172	trans-oleic acid (octadecenoic)	282	3.44	$C_{18}H_{34}O_2$
53.330	2196	stearic acid (octadecanoate of ethyl)	312	1.04	$C_{20}H_{40}O_2$
56.183	2300	Tricosane	324	1.61	$C_{23}H_{48}$

T = retention time; MM = molar mass; RA% = relative abundance; RI = retention index. Source: Prepared by the author (2025)

A mixture of compounds from different chemical classes, such as alcohols, aldehydes, ketones, and carboxylic acids constitute volatile oils. These are biosynthesized through the shikimic acid and mevalonate routes, during the secondary vegetable metabolism (Oliveira 2013).

The dominant compound 2,4,6-trimethyldecane has been identified in other aromatic species such as *Vigna radiata* (L.) Wilczek (Attar et al., 2017) and *Zingiber barbatum* Wall. (Shukurova et al., 2020); however, its presence in *Tridax procumbens*, a member of the Asteraceae family, highlights a broader distribution of this compound across taxonomic groups. Similarly, trans-oleic acid, an unsaturated fatty acid, while previously detected in species such as *Pseudostellaria heterophylla* (Miq.) and *Scrophularia ningpoensis* Hemsl. (Shen et al., 2008), may indicate shared metabolic pathways among diverse plant families, although its role in Asteraceae remains to be fully elucidated. (Miyazawa & Okuno 2003).

Citronellic acid or citronellol is a natural acyclic monoterpene present in species of the Asteraceae family, such as cardamom (*Elettaria cardamomum*), as well as roses (*Rosa* sp.), geraniums (*Chrysanthemum* sp.), and citronella. These compounds have applications as flavorings in food products and cigarettes. Studies have reported their repellent, larvicidal, and antibacterial properties, and also indicated that citronellol and its farnesyl derivatives can interfere with postprandial hyperglycemia. Research suggests that citronellol can be used in the treatment of dermatophytosis, mainly caused by *Trichophyton rubrum* (Pereira et al. 2015). Tetrahydrogeraniol is an acyclic monoterpene whose flavoring property is valued by the food industry (Valdes et al. 2019). Although its presence is not very common, according to Rahman (2017), it can be found in Amazonian species such as *Thevetia peruviana* (Pers.) K. Schum., and seems to contribute to its antifungal, anti-inflammatory, and insecticidal properties.

The trans-damascenone compound, a nor-isoprenoid, is identified in wine obtained from *Vitis vinifera*, and is responsible for its strong aroma (Yuan & Qian 2015). It is also one of the main compounds responsible for the aroma of fruits such as apricot, rose, starfruit, kiwi, mango, tomato, wine, rum, raspberry, passion fruit, and blackberry (Uenojo et al. 2007).

β-Atlantol is a monocyclic sesquiterpene present in the volatile oil of species such as *Piper aequale*. Studies by Fabri et al. (2019) and Bruni et al. (2004) showed its antibacterial properties in plants of the genus *Ocotea*. This compound was also detected in the leaves of *Begonia reniformis* (Silva et al., 2017) and in the flowers of *Xylopia aromatica* (Lam.) Mart., popularly known as "monkey pepper" (Nascimento et al. 2018).

α-Ionone, one of the major compounds in *T. procumbens* oil, was also detected in the volatile oil of *Dalbergia frutescens* (Vell.) Britton, a tree used in folk medicine for its antibacterial and anti-inflammatory properties (Mendes et al. 2012).

Therefore, considering the chemical profile presented and limiting the applicability of the compounds to species of the Asteraceae family, the volatile oil of *T. procumbens* is promising for investigations aimed at searching for aromas and condiments, as well as isolating compounds with antioxidant, antimicrobial, and toxic potential.

Toxicity Tests

The volatile oil of the *T. procumbens* inflorescence showed LC_{50} 1,079 $\mu g.mL^{-1}$ and was considered non-toxic to *A. salina*. According to the criteria employed by Amarante et al. (2011), natural compounds with LC_{50} higher than 1,000 $\mu g.mL^{-1}$ are considered non-toxic. We regarded this biological assay as a preliminary screening in studies of compounds with potential biological activities. Similar results were found with an aqueous decoction of the leaf and stem of *T. procumbens*, showing a LC_{50} result of 1,570 $\mu g.mL^{-1}$ (Koukoui et al. 2017).

The use of medicinal plants brings with it the concern with their toxicity, as the toxic effects presented by inappropriate use are often known or ignored. Plants can show toxicity according to their use, exposure time, form of preparation, among other ways. Thus, the toxicity of medicinal plants is a public health problem, as cases of poisoning resulting from their use are increasingly frequent (Bizzo et al. 2009).

Antimicrobial activity

The volatile oil of *T. procumbens* showed activity only against *S. aureus* ATCC 25923 with MIC of 250 μg.mL^{-1}. According to Holetz et al. 2002, compounds with MIC below 100 μg.mL^{-1} are considered as highly active against bacteria, those with MIC between 100 and 500 μg.mL^{-1} are considered to have moderate antimicrobial activity, those with MIC between 500 and 1,000 μg.mL^{-1} are weakly active, and those with a MIC above 1,000 μg.mL^{-1} are inactive. Table 2 shows the results found in our study with the volatile oil of *T. Procumbens*.

Table 2: Antimicrobial activity of the volatile oil of T. procumbens.

	Bacteria	MIC μg.mL^{-1}
Gram positive	*S. epidermidis* ATCC 12228	> 2,000
	S. aureus ATCC 25923	250
Gram negative	*P. aeruginosa* ATCC 27853	> 2,000
	K. pneumoniae ATCC 700603	> 2,000
	E. coli ATCC 25312	> 2,000

Source: Prepared by the author (2025)

Different extracts of T. procumbens were tested against four strains of bacteria, including E. coli, P. mirabilis, S. aureus, B. subtilis, and it was noted that the ethyl alcohol extract had the strongest inhibitory effect on all strains of bacteria (MIC = 25 - 10 mg.mL-1) (Andriana et al. 2019). The hexane extract inhibited E. coli and S. aureus, while the chloroform extract inhibited only S. aureus. The authors stated that chemical tests showed which substances such as stigmasterol, β-sitosterol and n-hexadecanoic acid were the main compounds in the fraction that showed the best antibacterial effect.

In another study, it was noted that stigmasterol and β-sitosterol isolated from T. procumbens had a broad spectrum of antibacterial activity. These sterols showed effective inhibition in S. aureus, S. albus, E. coli and Pseudomonas pyocyanea (Desbois & Smith 2010). Furthermore, (Shin et al. 2007) reported that n-hexadecanoic acid isolated from T. procumbens inhibited both Gram negative and Gram positive bacteria. Insertion of oily acid into the inner bacterial membrane increases the permeability of the membrane so that the inner content leaks out of the cell and induces growth inhibition or even death of the microorganism (Desbois & Smith 2010).

Conclusion

This study aimed to explore key floral morphoanatomical and chemical features of Tridax procumbens inflorescences, relevant for establishing pharmacognostic parameters and ensuring quality control of the raw material, especially given its use in Brazilian folk medicine.

In this research, we identified the main morphological inflorescence characteristics of T. procumbens that contribute valuable botanical data for the genus Tridax and the Asteraceae family. These morphoanatomical traits play an important role in quality control by helping to authenticate and standardize the raw plant material.

The chemical profile of the volatile oil extracted from the inflorescences revealed 21 compounds, mainly terpenes, including monoterpenes and sesquiterpenes, which are widely used in cosmetics, food, and medicinal applications.

The A. salina assay is a quick and cheap to be used to test bioactivity in natural products is exposure to it, ones that this saltwater microcrustacean is used as food for fish (Mclaughlin et al. 2016) and easily to find. Besides it, it's a good choice to test crude plant extracts aiming to determine the median lethal concentration (LC50) (Bednarczuk et al. 2010), in addition, this test does not require aseptic techniques, it is easy to perform and also, it can show relevant results.

The oil showed no toxicity to Artemia salina, supporting its safe use in folk medicine, while displaying moderate antimicrobial activity against Staphylococcus aureus, a significant pathogen.

Given these findings, further studies are warranted to better understand the chemical and biological properties of this species, including expanded antimicrobial testing against other strains of S. aureus and additional bacterial species.

Disclosure statement

The authors declare no conflict of interest.

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