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ANATOMY AND CHEMICAL CONSTITUENCY OF TREMA MICRANTHA FRUIT: RELATIONSHIP WITH DISPERSAL SYNDROME AND URBAN AFFORESTATION

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ABSTRACT

Purpose: This study aimed to analyze the anatomy and histochemistry of Trema micrantha fruit to correlate the data with its wide distribution in urban areas.

Theoretical framework: T. micrantha is a native tree species belonging to the Cannabaceae family and grows widely in urban areas, riparian forests, and environmental recovery areas in Brazil. The fruit is of the drupe type and is mainly consumed by avifauna, however, reports on its anatomy and chemical composition are lacking, and the relationship between fruit structure and dispersion syndromes has not been studied.

Method: The mature fruits were fixed, dehydrated, embedded, polymerized in methacrylate, and sectioned using a rotating microtome. The slides were mounted, stained, and analyzed under a light microscope. Surface analysis was performed using scanning electron microscopy (SEM).

Results and conclusion: The pericarp consisted of an exocarp, a mesocarp, and an endocarp. The exocarp is uniseriate and contained papillae, stomata, and a cuticle. The mesocarp is parenchymatic and subdivided into three regions: external, middle, and internal. The endocarp is uniseriate and contains sclerified cells. Histochemical tests revealed the presence of lipids, proteins, starch grains, phenolic compounds, and alkaloids in the mesocarp.

Research implications: The presence of lipids, proteins, and starch increases the nutritional value of the fruit, indicating its dispersion by animals, mainly birds, and the dispersion of diaspores in urban areas.

Originality/value: T. micrantha contributes to the food chain of local fauna, and their population will benefit from by increasing afforestation.

Keywords: Cannabaceae, Drupe, Histochemistry, Birds.

ANATOMIA E COMPOSTOS QUÍMICOS DO FRUTO DE TREMA MICRANTHA: RELAÇÃO COM A SÍNDROME DE DISPERSÃO E A ARBORIZAÇÃO URBANA

RESUMO

Objetivo: O objetivo do estudo foi analisar a anatomia e a histoquímica do fruto de *Trema micrantha* a fim de correlacionar a sua ampla distribuição em áreas urbanas.

Referencial teórico: *Trema micrantha* é uma espécie arbórea nativa, pertencente à família Cannabaceae. Ocorre amplamente no Brasil, em áreas urbanas, matas ciliares ou em áreas de recuperação ambiental. Possui fruto do tipo drupa apreciado pela avifauna, contudo, não há relatos na literatura sobre sua anatomia e composição química. Desse modo, faltam informações sobre a relação da estrutura do fruto e sua síndrome de dispersão.

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Método: Para isso, frutos maduros foram fixados, desidratados, infiltrados e polimerizados em metacrilato para cortes em micrótomo rotativo. Lâminas foram montadas, coradas e analisadas em microscópio de luz. Análises de superfície foram realizadas em microscópio eletrônico de varredura.

Resultados e conclusão: O pericarpo é constituído de exocarpo, mesocarpo e endocarpo. O exocarpo é unisseriado e possui papilas, estômatos e cutícula. O mesocarpo é parenquimático, subdividido em três regiões, que são: externo, médio e interno. O endocarpo é unisseriado com células esclerificadas. Os testes histoquímicos indicaram a presença de lipídios, proteínas, grãos de amido, compostos fenólicos e alcaloides no mesocarpo.

Implicações da pesquisa: A presença de lipídios, proteínas e grãos de amido aumentam o valor nutricional do fruto, indicando a dispersão por animais, principalmente por aves, o que por sua vez, favorece a dispersão dos diásporos em áreas urbanas.

Originalidade/valor: Desse modo, *Trema micrantha* é uma espécie que contribui para a alimentação da fauna local e ao bem-estar da população que será beneficiada com o aumento da arborização.

Palavras-chave: Cannabaceae, Drupa, Histoquímica, Pássaros.

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1 INTRODUCTION

Trema micrantha (L) Blume, locally called periquiteira, grandiúva, or pau-pólvora (POTT; POTT, 1994; GÓES; OLIVEIRA, 2011; MACÊDO *et al.*, 2012), is a pioneer species distributed across Mexico to South America (PEDERNEIRAS *et al.*, 2011). *T. micrantha* belongs to the Cannabaceae family in the Urticoide clade, can grow up to 20 m (VÁZQUEZ-YANES, 1998), and is dioecious or trioecious (VIANA; GIL, 2018). *T. micrantha* is an ecologically important species as it helps in forest recovery. It is a fast-growing tree with high fruit density and also a nitrogen-fixing species (ANDREANI *et al.*, 2014); it has drupe-type fruits (TORRE; LUCA, 2005) that are dispersed by bats, monkeys, and birds (KINOSHITA *et al.*, 2006). *T. micrantha* is also an economically important tree as it is used for beekeeping and foraging, and its wood is used for firewood and cellulose production (POTT; POTT, 1994). Studies also indicate its importance in popular medicine; the leaves and bark are used to prepare teas to combat syphilis and rheumatism and exhibit wound-healing properties through external washing (ALVES *et al.*, 2007).

T. micrantha is widely distributed in Brazil, found across 20 states (ANDREANI *et al.*, 2014), and is listed in urban afforestation inventories (SARTORI; BALDERI, 2011; BRANDÃO *et al.*, 2011; LINDENMAIER; PENA, 2014). It can grow in any environment, as the seedlings can tolerate open spaces with abundant sunlight and relatively poor soils (VÁZQUEZ-YANES, 1998). Therefore, it is commonly found in urban areas and is a part of the urban forests of some Brazilian cities, as many species of birds disperse the seeds(ANDREANI *et al.*, 2014; CAMARGO *et al.*, 2020).

The chemical composition of fruits can favor their dispersion by zoochory, especially birds, which are attracted to fruits with a high nutritional content (ESTRADA; FLEMING, 2012). Chemical and histochemical studies show that birds prefer three macromolecules: lipids, proteins, and carbohydrates, which are essential to their diet (ESTRADA; FLEMING, 2012). Anatomical and histochemical studies on fruits can help elucidate the structure of the pericarp and its chemical composition (BOBROV; ROMANOV, 2019), and this can help us further understand fruit-disperser relationships. However, studies on the fruit morphology of *T.*

micranta have not been performed; therefore, morphoanatomical studies are required to understand the histology and determine the main chemical compounds of the fruit.

This study aimed to analyze the structure and histochemistry of the fruit of *T. micrantha*, describe the parts and tissues that comprise the pericarp, and detect various chemical compounds present in the pericarp of the mature fruit. We intended to relate the structure and chemical composition of the fruit with dispersal syndrome and the occurrence of the species in urban afforestation inventories to verify the importance of including the species in urban afforestation plans.

2 METHOD

The mature fruits of *T. micrantha* were collected from Campo Grande in Mato Grosso do Sul and Ribeirão Preto in São Paulo state, Brazil. In Campo Grande, fruits were collected from trees located next to public roads (Figure 1) and from trees inside the Universidade Federal de Mato Grosso do Sul (UFMS) campus in Campo Grande. In Ribeirão Preto, fruits were collected from the University of São Paulo (USP) campus, next to internal roads. Vouchers of the collected material were deposited in the CGMS herbaria of Campo Grande (Voucher 81140), and the SPFP of the USP of Ribeirão Preto (Vouchers 16306 e 15958).



Figure 1 – Individual of *Trema micrantha* located on a public road in Campo Grande, Mato Grosso do Sul. a) Pistillate tree. b) Branch with green and ripe fruits.
Source: Authors.

For conserving and processing, the fruits were fixed in 50% formaldehyde, acetic acid, 50% alcohol, and neutral buffered formalin (JOHANSEN, 1940), dehydrated in ethanol with

increasing concentrations up to 70% alcohol, and stored for subsequent processing and analysis using a light microscope and scanning electron microscope (SEM). After fixing, the fruits were processed for softening (RIBEIRO; OLIVEIRA, 2014).

For anatomical analysis using light microscopy (LM), the material was dehydrated in increasing ethanolic series until 95% alcohol, infiltrated in methacrylate (Historesina - Leica®, Wetzlar, Germany) for at least one week, and then polymerized. Cross and longitudinal sections were performed using a Leica RM 2145 rotary microtome (5–9 µm). The sections were fixed on slides and stained with 0.5% toluidine blue in phosphate buffer pH 4.7 (O' BRIEN et al., 1964). The sections were analyzed, and photomicrographs were taken on a Nikon Eclipse Ci photomicroscope (Tokyo, Japan) with a Motic® Cam Pro 252B digital camera (Beijing, China).

For SEM observations, the samples were separated under a stereomicroscope, dehydrated in ethanol gradients, dried at the critical point in a Quorum Technologies type CPD7501 (Quorom, East Sussex, UK), mounted on metal supports on carbon adhesive tape, and sputtered with gold in a Denton Vacuum Sputter type Desk III (Denton, Moorestown, NJ, USA). Electron micrographs were obtained using a JEOL scanning electron microscope (JSM-6380LV, JEOL, Tokyo, Japan).

Histochemical tests were carried out to identify lipids with Sudan IV (PEARSE, 1985) and Oil red staining (PEARSE, 1968), proteins with Xylidine Ponceau staining (VIDAL, 1970), neutral polysaccharides with Periodic acid–Schiff (PAS) method (O'BRIEN; MCCULLY, 1981), starch with Lugol's iodine method (JOHANSEN, 1940), phenolic compounds with ferric chloride method (JOHANSEN, 1940) and alkaloids with Wagner's reagent (FURR; MAHLBERG, 1981).

3 RESULTS AND DISCUSSION

The mature fruit of *T. micrantha* has an ovoid shape and reddish color; its length varies from 5–6 mm (Figure 2a). The calyx and stigma persist in mature fruits (Figure 2a, b).

The fruit has three distinct parts: the exocarp (Figure 2c, d), mesocarp (Figure 2d), and endocarp (Figure 2e). The surface of the fruit (exocarp) contains papillae and stomata (Figure 2c, f). The exocarp is uniseriate, with cuboidal cells and a cuticle (Figures 2d, f, 3a). The mesocarp is parenchymatic and may be subdivided into the outer mesocarp, formed by three to four layers of cells with druses; the medium mesocarp has several layers, large and elongated cells with druses and vascular bundles (Figure 2d, g); and the inner mesocarp consists of four to five layers of rounded and compressed cells (Figure 2d, g). The endocarp with fully lignified cells without a visible lumen comprises macrosclereids (Figure 2e, h).

As a result, *T. micrantha* has a drupe fruit confirmed by its anatomical structure and corroborated by the morphological work of the species (TORRES and LUCA, 2005). This is the first anatomical and histochemical analysis of the fruit of *T. micrantha* and one of the first studies on fruit morphoanatomy in the Urticoide clade consisting of Cannabaceae, Moraceae, Ulmaceae, and Urticaceae families (SYTSMA et al., 2002). The pericarp is characterized by a uniseriate exocarp, a fleshy parenchymatic mesocarp, and a rigid endocarp attached to the seed (present study), as described for drupes of other species (SOUZA, 2006).

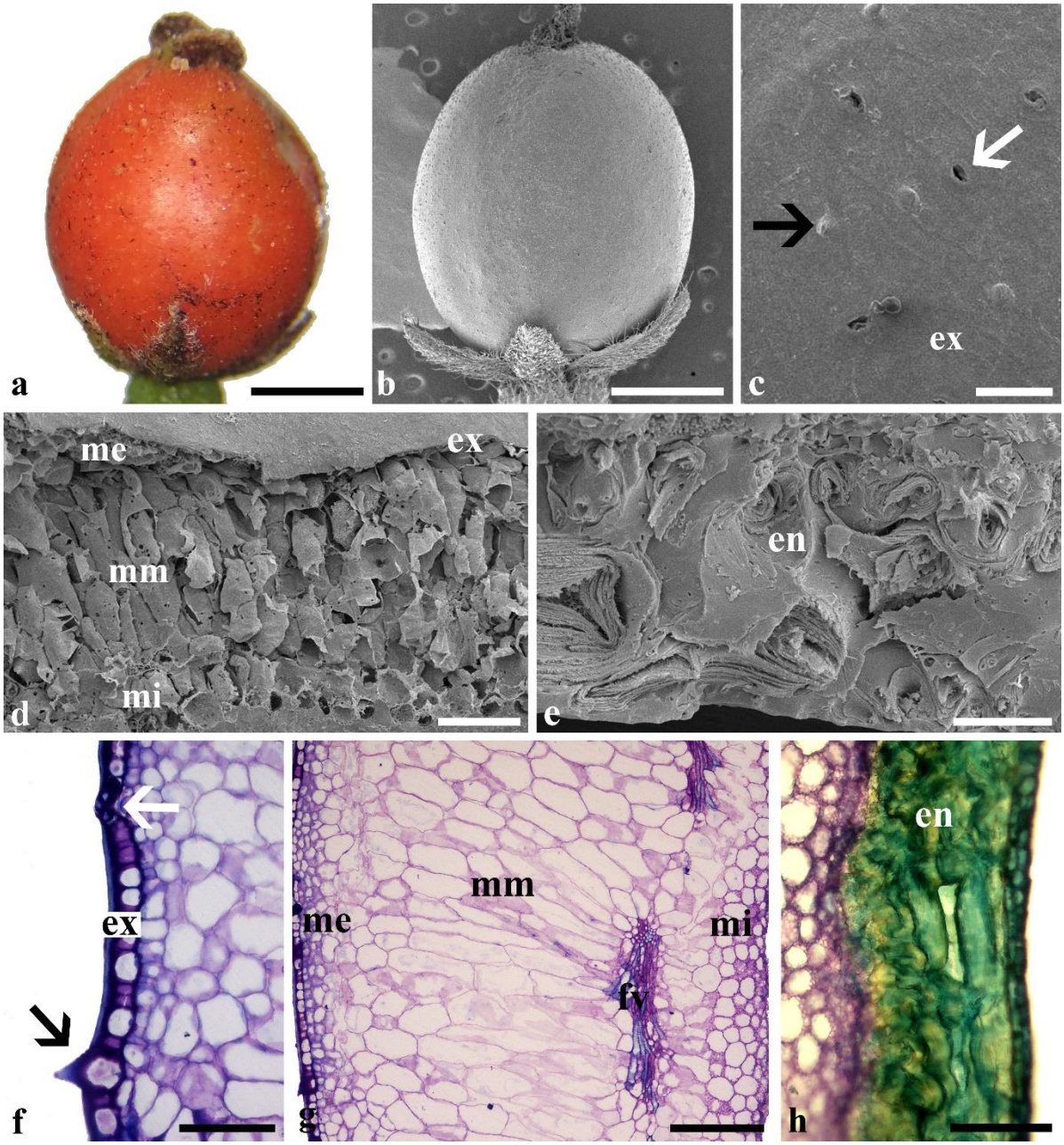


Figure 2 – Mature fruit of *Trema micrantha* and details of the pericarp (a), SEM (b-e), and light microscopy (f-h). a) Fleshy mature fruit. b) Electromicrograph of the mature fruit. c) Surface of the exocarp with papillae (black arrow) and stomata (white arrow). d) Uniseriate exocarp. Outer mesocarp (me), middle mesocarp (mm), and inner mesocarp (mi). e) Endocarp (en) with lignified cells and reduced lumen. f) Uniseriate exocarp (ex) with papillae (black arrow) and stomata (white arrow). g) Multiseriate mesocarp, subdivided into outer (me), middle (mm), and inner (mi) mesocarp, with vascular bundles. h) Endocarp (en) with lignified cells stained green with toluidine blue. Scales: (a, b) 2 mm, (c, d, g) 200 µm, (e) 40 µm, (f, h) 100 µm.
Source: Authors.

Histochemical tests revealed the presence of starch, lipids, proteins, alkaloids, and phenolic compounds (Figure 3a-g, Table 1). A cuticle that reacted positively to Oil Red O stain was identified in the exocarp (Figure 3a). Starch grains were detected with Lugol's iodine in the guard cells of the stomata (Figure 3b). In the mesocarp, lipids were detected by Oil Red O stain (Figure 3a) and Sudan Black (Figure 3e) and reacted with Lugol's iodine indicating presence of starch (Figure 3c). Furthermore, protein bodies were detected with Xylidine

Ponceau (Figure 3d), alkaloids were detected with Wagner's reagent (Figure 3f), and phenolic compounds were detected with ferric chloride (Figure 3g).

The presence of lipids, proteins, starch grains, phenolic compounds, and alkaloids in the pericarp tissues is directly related to fruit dispersal syndrome by birds (ESTRADA; FLEMING, 2012), contributing to the presence of the species in inventories of urban afforestation (see SARTORI and BALDERI, 2011; BRANDÃO *et al*., 2011; LINDENMAIER and PENA, 2014).

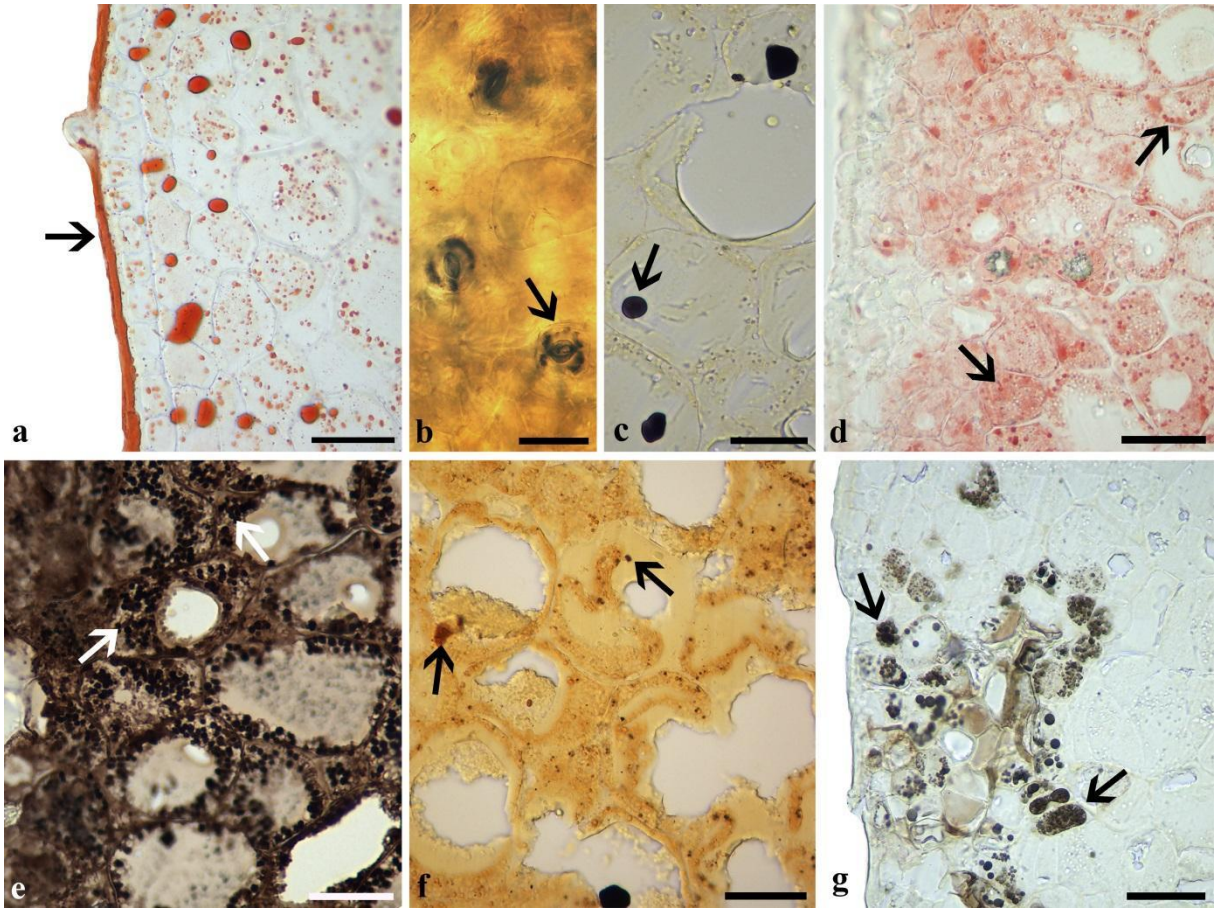


Figure 3 – Histochemical tests performed on the pericarp of the mature fruit of *Trema micrantha* in a cross-section (a,c-g) and paradermal section (b) (ML). a) Oil Red O test indicated lipids in the exocarp cuticle (arrow). b) Lugol's iodine test detected starch grains in the guard cells of the stomata in the exocarp (arrow). c) Lugol's iodine detected starch grains in the mesocarp (arrow). d) Xylidine Ponceau detected proteins in the mesocarp. e) Lipid reacted with Sudan black in the outer and middle mesocarp. f) Alkaloids were detected with Wagner's reagent. g) Phenolic compounds were detected with ferric chloride in the exocarp and outer mesocarp. Scales: (a-g) 50 µm.
Source: Authors.

Table 1 - Histochemical data obtained for the pericarp of the mature fruit of *Trema micrantha*.

Reagent	Target Compound	Mature Fruit
Periodic acid–Schiff	Neutral polysaccharides	-
Lugol's iodine	Starch grains	+
Sudan Black B	Total lipids	+
Oil red O	Total lipids	+
Xylidine Ponceau	Protein	+
Wagner's reagent	Alkaloids	+
Ferric chloride	Phenolic compounds	+

Source: Authors.

The mature fruit of *T. micrantha* has a slender exocarp, cuticle, and papillae. The cuticle was strongly evident after reaction with Oil Red (Figure 3a), also observed in *Brosimum gaudichaudii* Trécul fruits (Moraceae - JACOMASSI *et al*., 2010). Thus, the glossiness of the mature *T. micrantha* fruit is likely due to the presence of the cuticle. Cuticle regulates evapotranspiration, plays a role against mechanical stress (YEATS *et al*., 2012), and protects against pathogens (DOMÍNGUEZ *et al*., 2011). Characteristics that favor maintaining fruits on branches for longer periods may provide greater consumption by the dispersing agent.

The mesocarp can be divided into three different regions based on the size of its cells: the outer, middle, and inner mesocarps, as previously described for other drupes (SOUZA, 2006). These three parts are made up of parenchyma, which makes the fruit fleshy, and adds to the thin exocarp; thus, it may be considered a fruit that is easily accessible to the local fauna. Lipids, starch grains, and proteins in *T. micrantha* fruit make it more palatable and nutritious to animals (CORLETT, 1996). Moreover, the edible part of the fruit is the mesocarp, unlike in some Moraceae species whose edible part is formed from parts of the inflorescence (for example, *B. gaudichaudii*: inflorescence receptacle and *Maclura tinctoria*: axis of the inflorescence) (JACOMASSI *et al*., 2010; OYAMA; SOUZA, 2011).

The endocarp of *T. micrantha* is completely lignified and consists of a layer of sclerenchymatic macroscleid cells that protect the seed. Macrosclereids that form pyrenes are cited as drupes present in the infructescences of *M. tinctoria* (OYAMA; SOUZA, 2011) and *B. gaudichaudii* (JACOMASSI *et al*., 2010) of Moraceae.

Laticifers were not found in the pericarp of *T. micrantha*, although they were recorded in the ovaries of the same species and three other genera of Cannabaceae, *Cannabis*, *Celtis*, and *Pteroceltis* (LEME *et al*., 2020). These data suggest that laticifers may have been suppressed during the development of the ovarian wall to form the fruit. The function of laticifers is to defend against herbivory (LEME *et al*., 2020), and in mature fruits, such a defense is no longer necessary, considering that the dispersion syndrome of *T. micrantha* fruits is mainly caused by animals.

Phenolic compounds and alkaloids were detected in the histochemical tests, suggesting the phytotherapeutic potential of *T. micrantha* fruit. They may have the same chemical constituency as the leaves with analgesic and anti-inflammatory effects (BARBERA *et al*., 1992). The hypoglycemic effect of the extract of *T. micrantha* leaves has also been described by isolating the alkaloids and flavonoids (phenolic compounds) (SCHOENFELDER *et al*., 2006). Our findings reinforce the importance of studying the chemical profiles of *T. micrantha* compounds, mainly because they are used in popular medicines (ALVES *et al*., 2007).

Furthermore, the presence of cannabinoids in the inflorescences of *Trema orientalis* (L.) Blume (NAPIROON *et al*., 2021) and *T. micranta* (TEIXEIRA *et al*. 2023) are notable as these compounds are widely known and described from *Cannabis sativa* for their medicinal effects, further indicating the presence of cannabinoids in other species of the family.

Zoochory is the primary method of seed dispersal in *T. micrantha*, and birds (ornithochory) predominate in studies on seed dispersal (ANDREANI *et al*., 2014). The fruits of *T. micrantha* are highly sought after by different species of birds across different regions because of the density of fruits provided by the plant (ANDREANI *et al*., 2014). The lipid content of the fruit that is observed in this study suggests that it is consumed mainly by frugivorous birds as they prefer fruits with high-fat content that can provide them with high calories (BOROWICZ; STEPHESON, 1985). The presence of proteins in the mesocarp further contributes to the dispersion of *T. micrantha* fruit (ESTRADA; FLEMING, 2012). The bird–plant relationship enhances the dispersal of *T. micrantha* diaspores, contributing to ecological succession in degraded areas undergoing recovery. *T. micrantha* is a pioneer species widely

used in reforestation (ANDREANI et al., 2014; VASQUEZ-YANES, 1998), contributing to the conservation of native species (POZZETTI, 2020).

Thraupidae is the main family of birds that disperse the fruits of *T. micrantha* (ANDREANI *et al*., 2014, CAMARGO *et al*., 2020). Thraupidae is an endemic omivorous bird family in the Neotropics, and the morphotype of the animals is highly varied. They feed mainly on insects and fruits and can be found in open areas as well as closed vegetation (WIKIAVES, 2022). The wide distribution of *T. micrantha* coincides with the distribution of Thraupidae in the Neotropics. Therefore, the distribution of *T. micrantha* is largely due to the distribution of the Thraupidae species, which act directly in the dispersal of fruits. Hence, the consumption of *T. micrantha* fruit by Thraupidae species is of extreme ecological importance for both the tree and the birds.

Consequently, the fruits of *T. micrantha* are attractive nutritional resources for birds, favoring the dispersal of their diaspores in urban areas. *T.micrantha* is a native species and is highly recommended as a potential species for urban afforestation (MACÊDO *et al*., 2012; TAKEDA, 2013; XIMENEZ, 2018). Species diversification is an important aspect of afforestation plans; therefore, using native species favors diversity and avoids problems regarding growth, adaptability, and development, as they are adapted to local characteristics (XIMENEZ, 2018). Therefore, *T, micrantha* is generally present in urban inventories because of its fast-growing nature that adapts to rich and impoverished soils (VÁZQUEZ-YANES, 1998). Therefore, we suggest implementing this species in urban afforestation plans because of its rapid growth capability, which favors shade provision and provides food for local birds.

Studies such as this are important to encourage effective public actions for the conservation of native species, such as *T. micranta*, like other studies that encourages effective actions by authorities to interrupt environmental degradation processes (KOCH *et al*., 2023).

4 CONCLUSION

The fruit of *T. micrantha* is a drupe with a parenchymatic mesocarp and contains lipids, starch grains, and proteins that favor the dispersal of diaspores by birds. This likely contributes to the distribution of this species in urban areas, as observed in the present study and in surveys of urban afforestation. Although it is not widely used in afforestation plans, *T. micrantha* has been cited in previous studies as a potential native species for urban afforestation. Thus, the species studied here can be considered an important native species for maintaining urban forests, and as it is a pioneer species, it favors the emergence of secondary species in recovering forest fragments. Future studies on the ontogeny of the fruits of *T. micrantha* and other species of Cannabaceae are needed to understand the nature of the parts of the pericarp and the taxonomic relationships in the group.

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