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Pharmacological Potential of *Tetradenia Riparia* Plant and its Derivatives: A Scoping Review

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Pharmacological Potential Of *Tetradenia Riparia* Plant And Its Derivatives: A Scoping Review

Trabalho Conclusão do Curso de Graduação em Farmácia do Centro de Ciências da Saúde da Universidade Federal de Santa Catarina como requisito para a obtenção do título de Bacharel em Farmácia
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Pharmacological Potential Of *Tetradenia Riparia* Plant And Its Derivatives: A Scoping Review

Este Trabalho Conclusão de Curso foi julgado adequado para obtenção do Título de "Bacharel" e aprovado em sua forma final pelo Curso de Farmácia

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Dedico este trabalho a todos os cientistas, que nestes momentos obscuros encontrem luz para continuar lutando a favor da humanidade

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"I appreciate all the difficulties I faced; if it weren't for them, I wouldn't have moved. The facilities prevent us from walking. Even criticism helps us a lot."

(Chico Xavier)

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SUMÁRIO

Pharmacological potential of *Tetradenia riparia* plant and its derivative: a scoping review

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HIGHLIGHTS

- *Tetradenia riparia* have been obtained from subtropical areas, such as South Brazil.
- *T. riparia* essential oil has a potential pharmacological action on pathogens.
- TrEO and TrROY showed antibacterial, antifungal and antileishmanial activity .
- *T. riparia* showed immunomodulatory, antioxidant and anticholinesterase effects.

ABSTRACT

This scoping review to raise the pharmacological potential of the plant and its derivatives for health. We conducted this study according to PRISMA recommendations and registered in OSF (doi:10.17605/OSF.IO/3QFBG). We searched in three databases using the free terms combined with boolean operators: *Tetradenia riparia* OR *Iboza* OR *Moschosma*. PICOS strategy was applied to formulate the search question, eligibility criteria (inclusion and exclusion) and data extraction. Two independent and blinded reviewers conducted the selection and data extraction phase, and an expert removed the discrepancies. A total of 24 studies were included. The leaves and essential oils were majority studied, following crude extracts and fractions or isolated compounds. A plant was obtained in subtropical regions, mainly South of Brazil. Antimicrobial activity was reported for some in vitro studies, as antileishmanial, anti-tuberculosis bacteria, and helminthic action. Also, *T. riparia* showed antioxidant effects, acaricidal and immunomodulatory. The therapeutic use of this plant and its derivatives to treat infectious and inflammatory diseases was potential, but limited since regarding the cytotoxicity and few studies in vivo and humans published.

KEYWORDS: Lamiaceae, volatile Oils, Plants Oils, Cultured cells, Plant Extracts, Pharmacology.

1. INTRODUCTION

The plant *Tetradenia riparia* is a shrub of the Lamiaceae family with a size between one meter and a half to two meters in height. It is a very floriferous tree, with flowers of white and lilac colour, it can even be pink, with a pleasant perfume and attracts many pollinating insects. Its leaves are thick, oval to cordiform, pubescent, light green in colour, jagged margins and quite aromatic (Patro, 2016). This plant is grown in subtropical and temperate areas. It is found in Brazil, Africa and Asia as an ornamental plant in gardens, parks, houses, and mainly due to its intense aroma. Due to its pharmacological properties, it is used in the treatment of infectious and inflammatory diseases in traditional African and Asian medicine. *T. riparia* is also known as Iboza riparia and *Moschosma riparium* (Martins *et al.* 2008).

The essential oils (EO) that are most investigated belong to the years, and are obtained from leaves. This plant contains a complex of terpenoids, including monoterpenes, sesquiterpenes and diterpenes (Gazim *et al*. 2010; Demarchi *et al.* 2015a, 2016). In addition, they can be found in plant extracts α-pyrones and phenolic compounds. The percentages of the isolates can vary depending on the season and also where they were grown. An example of this is when sown in Brazil has a higher amount of oxygenated sesquiterpenes in the extracted essential oil than those grown in Africa. The most commonly found compound is monoterpene (Cardoso *et al*. 2015).

The pharmacological potential of *T. riparia* has been extensively studied, and its properties of interest in different diseases. Within them the ability to destroy the bacterium that causes tuberculosis, *Mycobacterium tuberculosis* (Baldin *et al.* 2018), anti-*Leishmania* action (Demarchi *et al.* 2015a; Cardoso *et al.* 2015; Demarchi *et al*., 2016; Terron-Monich *et al.* 2019), anthelmintic activity (Van Puyvelde *et al.* 2018), antioxidant (Friedrich *et al*. 2020), analgesic and antimicrobial (Gazim *et al*. 2010) and immunomodulatory (Demarchi *et al.* 2015b).

Some studies on *T. riparia* have been published in recent years addressing different therapeutic purposes (Gazim *et al.* 2010; Van Puyvelde *et al.* 2018; Friedrich *et al.* 2020; Demarchi *et. al.* 2015a, 2015b, 2016; Terron-Monich *et al.* 2019; Baldin *et al.* 2018; Endo *et al.* 2015 and 2018), it is necessary to synthesize the main studies on the pharmacological actions of this plant to guide its safe and effective use. In this study, we conducted a scoping review to synthesize the results of the main studies in the scientific literature on the pharmacological actions of the *T. riparia* plant and its derivatives.

2. MATERIAL AND METHODS

2.1 Study design

This scoping review followed the methodological recommendations for this type of study (Colquhoun *et al.* 2014; Levac *et al.* 2010; ArKsey and O'Malley 2005) and Preferred Reporting Items for Systematic reviews and Meta-Analyzes extension for Scoping Reviews (PRISMA-ScR) Checklist (Tricco *et al*. 2018). It was registered as the study protocol in the *Open Science Framework* (OSF, www.osf.io, Center for Open Science, Reino Unido, doi:10.17605/OSF.IO/3QFBG). The formulation of the scientific question was formulated based on the acronym PICOS (population, intervention, comparison, outcome and study design (What are the major pharmacological and toxicological effects of the plant *Tetradenia riparia* and its derivatives?) (Needleman, 2002; Higgins e*t al.* 2011) (Supplemental file Table S1). Also, other four stages were carried out: Identification of relevant studies; Study selection; Data extraction and Synthesis of data.

2.2 Identification of relevant studies

The studies were searched in databases based on systematic research in three databases: PubMed, LILACS and Google (grey literature), from November to March 2021. The search included the Boolean operators (AND/OR) and keywords/descriptors for the plant *Tetradenia riparia* (Supplemental file Table S2). As a search strategy, we used the free terms: *Tetradenia riparia* OR *Iboza* OR *Moschosma* in the different databases. A previous survey (pilot) was carried out to ascertain the potential Medical Subject Headings (MeSH) descriptors from PubMed / Medline and Health Sciences Descriptors (DeCS / Latin American and Caribbean Science Information Center) of health). As a pilot, we conducted an exhaustive search on PubMed using the descriptors, but this resource did not recover the potential articles and also raised many unimportant studies. Therefore, we retrieved the studies in the databases using only the free term and no date or language restrictions were applied to the initial search. The search in LILACs was done via the iAH form using: lamiaceae [Subject descriptor] AND NOT review [Words]. On google, the strategy applied was ‘allintitle: tetradenia riparia’ (Supplemental file Table S2). All articles identified in the research were transferred to a reference management software (My Web EndNote, Thomson Reuters) and all duplicates were removed, and then we carried out the study selection step.

2.3 Study selection

The PICOS structure (population, intervention, comparator, results and study design) (O'Connor, Green and Higgins, 2008) was used to establish the inclusion and exclusion eligibility criteria (Supplemental file Table S1). Original studies (*in vitro*, *in vivo* and *in human*) were included. Articles in English, Portuguese and Spanish with abstracts available were selected, and the year of publication of the study was not applied. Articles in the form of a letter, conference, editorial or guidelines, conference abstract, and reviews were excluded.

The first stage of the selection was carried out by two students (Group I) who reviewed the titles and abstracts independently and blindly (CLC, TP). In cases of disagreement, two expert reviewers (JV and IGD) determined the final inclusion of the study. The second stage for the selection of articles was carried out from the reading of the studies in full text (PDF) by the students. In case of disagreement, again, the experts entered into a consensus to determine the final inclusion. The selection step was carried out in the Rayyan QCRI mobile and web application (Ouzzani *et al.* 2016).

2.4 Data extraction and synthesis

After selecting the articles, the following data was recorded in an standard Excel spreadsheet: author (s), year, country, study objective (s), type of study, statistical analysis, experimental model, part of the plant, concentration or dose of active compound studied, time of treatment, pharmacological effect and conclusions. The articles were randomly distributed to two reviewers blindly (Research Randomizer® version 4.0 computer software, Urbaniak and Plous, 2013). And the reviewers extracted the data independently. Group I checked the data in pairs. After consensus and corrections, the articles were also randomly distributed to the experts. This step was done to validate the extracted data in pairs and thus guarantee the accuracy and quality of the review. A narrative synthesis of the results was carried out and according to the pharmacological potential and the type of study. Until this synthesis, we have not finalized a risk of bias assessment. Thus, we have not reported it in the present study (in progress).

3. RESULTS

In this scoping review, we identified 111 potential studies in three databases, after removing duplicates (4), 107 articles were transferred to read the title and abstract (Figure 1). In this phase, we excluded 77 studies (49 wrong interventions, 15 publication types, wrong outcome (10), and other reasons. And one study was recovered by topic expertise. After, in the full-text eligibility phase, we readed 31 studies, selecting a total of 24 articles for qualitative synthesis. We excluded six studies since they did not attempt the PICOS criteria (3 wrong outcomes, 3 wrong publication type, and one wrong population (Figure 1, and Supplemental file S3).

3.1 Summary of characteristics of the included studies

The most of the studies included in this review (13/24) were carried out in Brazil (Table 1) (Baldin *et al.* 2018; Cardoso *et al.* 2018; Demarchi *et al.* 2015a, 2015b, 2016; Endo *et al.* 2015; Fernandez *et al*. 2017; Gazim *et al.* 2010, 2011, 2014; Melo *et al*. 2015a, 2015b; Terron- Monich *et al.* 2018). The most the plant source reported was the Brazilian South region, especially Paraná state, and mainly in the city of Umuarama located in the northwest of the state (Table 1) (Baldin *et al.* 2018; Cardoso *et al.* 2018; Demarchi *et al.* 2015a, 2015b, 2016; Endo *et al.* 2015; Fernandez *et al*. 2017; Gazim *et al.* 2010, 2011, 2014; Terron- Monich *et al.* 2018). São Paulo was highlighted as another state of Brazil that was collected near Franca city for studies by Melo *et al.* (2015a, 2015b). Other countries were reported as *T. riparia* source as South Africa (4/24, Amoo *et al.* 2012; Okem *et al.* 2012; Sabela *et al.* 2017; York, Van Vuuren and Wet, 2012), Kenya (3/24, Nidiku and Ngule, 2014; Ngule *et al.* 2014; Omolo *et al.* 2004), Rwanda (2/24, Van Puyvekde *et al.* 1986, 2018), Uganda (1/24, Kakande *et al.* 2019) and Democratic Republic of the Congo (1/24, Ngbolua *et al.* 2016).

All studies used the leaves to conduct the experiments (Table 1). Fresh leaves extracts (Amoo *et al*. 2012), essential oils (Baldin *et al.* 2018; Cardoso *et al.* 2018; Demarchi *et al.* 2015a, 2015b, 2016; Gazim *et al.* 2010, 2011, 2014; Melo *et al.* 2015a, 2015b; Omolo *et al.* 2004; Terron-Monich *et al.* 2018), and isolates compounds. These were tested in experimental studies. The isolates compounds tested were 6,7-dehydroroyleanone (Baldin *et al*. 2018; Demarchi *et al.* 2015a and Gazim *et al.* 2014; Terron-Monich *et al.* 2018), 8(14),15-Sandaracopimaradiene-7 alfa,18-diol was isolated by Van Puyvelde *et al.* (1986 and 2018). Gazim *et al.* (2014) isolated 29 fractions of

essential oil, but just fraction 16 (9β,13β-epoxy-7-abietene) and 17 (6,7-dehydroroyleanone) showed the potential pharmacologic .

Also, other derivatives were tested as hydroalcoholic extracts (Endo *et al.* 2015), crude extract (Fernandez *et al.* 2017; Kakande *et al.* 2019; Ndiku and Ngule, 2014; Ngbolua *et al.* 2016; Okem, Finnie and Van Staden, 2012, Sabella *et al.* 2017) and fractions as FR-I (abieta-7,9(11)-dien-13β-ol), FR-II (Ibozol), FR-III (8(14), 15-sandaracopimaradiene-2alfa,18-diol and 8 (14), 15-sandaracopimaradiene-7alfa, 18-diol), and FR-IV (Astragalin, Boronolide and Luteolin) (Fernandez *et al.* 2017). After the phytochemical screening, the compounds identified were the major tannins, phenols, glycosides and terpenoids.

Gazim *et al.* (2010) identified a varied yield of TrEO according to the climatic season. The largest TrEO extraction was found during winter (0.265% ± 0.0), decreasing significantly in spring to 0.168% ± 0.02. Also, they highlighted that during the spring, the rainfall was much higher than in the other seasons in those regions. The seasonal influence on the TrEO yield was also described by Cardoso *et al.* (2015). They also studied the TrEO obtained from different seasons on leishmaniasis *in vitro* and *in vivo* experiments (described below).

3.2 Pharmacological potential

Twenty-three studies were conducted *in vitro* (Amoo *et al.* 2012; Baldin *et al.* 2018; Cardoso *et al.* 2018; Demarchi *et al.* 2015a, 2015b and 2016; Endo *et al.* 2015; Fernandez *et al.* 2017; Gazim *et al.* 2010, 2011 and 2014; Kakande *et al.* 2019; Melo *et al.* 2015a and 2015b; Ndiku and Ngule 2014; Ngbolua *et al.* 2016; Ngule *et al.* 2014; Okem, Finnie and Van Staden, 2012; Sabela *et al.* 2017; Terron-Monich *et al.* 2018; Van Puyvelde *et al.* 1986 and 2018; York, Van Vuuren and Wet, 2012) and two were realized studies *in vivo* too (Cardoso *et al.* 2018; Gazim *et al.* 2010) and one *in human* (Omolo *et al*,. 2004) (Table 1). *T. riparia* and derivatives have shown potential pharmacological action on a variety of pathogens, insects and cells (Table 2). We provided a narrative synthesis according to pharmacological assays.

3.2.1 Antioxidant and cytotoxicity

The cytotoxicity activity was reported by nine *in vitro* studies (Baldin *et al.,* 2018; Cardoso *et al.* 2018; Demarchi *et al.* 2015a; Demarchi *et al*. 2015b; Demarchi *et al.* 2016; Gazim *et al.* 2014; Melo *et al.* 2015b; Ngbolua *et al.* 2016; Okem, Finnie and Van Staden, 2012; Sabela *et al.,* 2017) (Tables 1 and 2).

TrEO was cytotoxic for murine macrophages in three studies (~CC50 3 mg/mL) (Baldin *et al*. 2018; Cardoso *et al*. 2018; Demarchi *et al.* 2015a), and two showed that TrROY had a high potential for toxicity in murine macrophages (CC50 <1 mg/mL) (Baldin *et al.* 2018; Demarchi *et al.* 2015a). Regarding murine cytotoxicity, TrEO (Cardoso *et al.* 2018; Demarchi *et al.* 2015a) and TrROY did not show erythrocyte toxicity potential (Demarchi *et al.* 2015a). Melo *et al.* (2015b) observed that TrEO at 200 µg/mL was cytotoxicity on Chinese hamster lung fibroblast cells (V79) concentration.

Baldin *et al.* (2018) evaluated *in vitro* cytotoxicity of TrEO and TrROY on murine peritoneal macrophages from male BALB/c mice using Alamar Blue assay (Mikus and Steverding, 2000) (Table 1). TrEO and TrROY were added from (from 0.39 to 100 µg/mL), and the outcome was expressed in 50% cytotoxicity concentration (CC50) and selectivity index (SI) (Baldin *et al.* 2018). CC50 of TrEO was 122 µg/mL and SI 1.9, and CC50 TrROY was 247 µg/mL and SI 7.9 (Table 2). Remembering that SI was determined by CC50/ MIC of *Mycobacterium tuberculosis* assay. TrROY showed less cytotoxicity for murine macrophages than TrEO, which is acceptable, since it is a mixture of compounds (Baldin *et al.* 2018; Demarchi *et al.* 2015a)

Cardoso *et al.* (2018) using Alamar Blue assay, tested TrEO obtained from different seasons (from 0.002 to 0.2 µg/mL). CC50 was about 90 ng/mL for summer and autumn EOs. The SI was measured by comparison between CC50 and antileishmanial promastigote assay, and being the major seasons showed SI above one (values below 1 are considered more toxic to the host), and the best result was obtained with the summer extraction (6.01). They also tested the cytotoxicity on macrophages J774.A1 using the XTT colorimetric assay (2,3-Bis[2-methoxy-4-nitro-5-sulfopheny]-2H-tetrazolium-5-carboxinilide, (XTT) (TrEO tested at 4,800, 480, 300, 30, 3, or 0.3 ng/mL). The highest result of CC50 was for summer TrEO (1476 ng/mL) and autumn the lowest value (391.66 ng/mL) (Cardoso *et al*. 2018). Similar results were showed by Demarchi *et al.* (2015a).

The lower or absent erytrocyty toxicity activity of TrEO and TrROY was described by Demarchi *et al.* 2015a and Cardoso *et al*. 2018. Basically, fresh defibrinated human blood was obtained, TrROY and TrEO was dissolved in dimethylsulfoxide (DMSO, concentration not cytotoxicity). Then, a serial dilution in

plates was performed, where the concentration for TrEO was 5.0 to 0.1 µg/mL and for TrROY 50 to 0.1 µg/mL, after being incubated with the erythrocytes. The percentage of hemolysis that occurred was determined after 2h incubation, and the result obtained was ~3% hemolysis at 5 µg/mL of TrEO and at 50 µg/mL of TrROY. It was less than 18% shown by the reference drug amphotericin B (AmB). Cardoso *et al.* (2018) tested TrEO obtained from different seasons, and showed that at the highest concentration (2.4 µg/mL), TrEO causes less hemolysis than AmB. Emphasizing the differences between the period of the year, in summer it had the lowest potential for hemolysis (0.63%), while in autumn the highest (4.01%) (Cardoso *et al.* 2018).

In the studies of Demarchi, CC50 TrROY was 0.53 µg/mL on murine macrophages, showing 0.22 of SI (therapeutic selectivity index, TSI was 0.03). For TrEO CC50 was 0.17 µg/mL, resulting in 5.67 for SI and 0.34 for TSI. In another study with TrEO, Demarchi *et al*. (2015b) used two methods to identify cytotoxicity in murine macrophages, one of which was the same performed in the previous study, where it presented a very close result for CC50 (165 ng/mL). In the three trials (Demarchi *et al.* 2015a; 2015b and 2016) the results presented for this test were very close.

Another method used (Demarchi *et al.* 2015b) was expressed by the percentage of cell viability using Trypan Blue assay. It was based on a 24-well plate culture, containing a suspension of macrophages, after washing different concentrations of TrEO were added from 3 µg/ml to 30 ng/ml. This plate was incubated and then stained with 1% Trypan Blue and examined under a microscope. The result of this test was quite satisfactory, as approximately 90% of the cells remained viable, when compared to untreated cells, which were 96% (Demarchi *et al.* 2015b).

Melo *et al*. (2015b) conducted a study to discover the antischistosomal activity and also the cytotoxicity of plant EO. Chinese hamster lung fibroblast cells (V79) were tested using the same cytotoxicity method for murine macrophages mentioned above (Demarchi *et al.* 2015a; 2015b; 2016) . The TrEO concentration used was 3.12 to 400 µg/mL dissolved in DMSO. This study showed that the concentration of 200 µg/mL was toxic for this type of cell.

In this review, other studies tested extracts from *T. riparia* leaves. A selection of plants used in South Africa for diseases related to the stomach, was reported by Okem, Finnie and Van Staden, (2012), that performed extracts in ethyl acetate, ethanol and water from the leaves of the *T. riparia* plant. The method to assess genotoxicity, done to guarantee the safety of the plant's therapeutic use, was with the *Salmonella* microsome assay, called the Ames test. The result was that none of the extracts

showed to be mutagenic agents, but it is important to remember that in this test there is no metabolic activation, which implies that the biotransformation of the compounds can be mutagenic when tested *in vivo.*

Gazim *et al.* (2014) the cytotoxic activity was evaluated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide, MTT) method (Berridge *et al.* 1996). The assay was carried out using 4 different human tumor cell (MDA-MB-435/breast tumor, HCT-8/colon, SF-295/nervous system, and HL- 60/promyelocytic leukemia). TrEO showed high proliferation inhibition for SF-295 tumor cells (78.06%) and HCT-8 cells (85.00%). Fraction 16 also showed high inhibition activity 94.80% and 86.54% for SF-295 and HCT-8 tumor cells, respectively. And for MDA-MB-435 strain both showed low activity. On the other hand, fraction 17 (TrROY) did not show any activity in the cells studied. (Gazim *et al.* 2014). Sabela *et al.* (2017), used silver nanoparticles (AgNPs) from the aqueous extract of the plant leaves. Using the MTT method and as an embryonic kidney cell population. They maintained at a concentration of 400 µM, the viability of cells at a percentage of 49.7%, above that concentration, it became toxic for the cells studied (Sabela *et al.* 2017).

One of the articles studied the pharmacological potential for the treatment of sickle cell anaemia (Ngbolua *et al.* 2016). The antisickling capacity of the leaf extract was tested. Five antisickling and hemolytic experiments were performed. One of the tests was called EMMEL, using blood from patients treated at the "Centre de Médecine Mixte et d'Anémie SS", located in Kinshasa, DR Congo, performing haemoglobin electrophoresis to confirm the nature of SS. With that, an aliquot of the blood was diluted in saline mixed with sodium metabisulfite, and it was added a drop of this mixture on a microscope slide with, or not organic acids extract. Finally, after sealing the slide to exclude air, the results were analyzed by software. Microscopy showed that treatment with organic acids restored the sickle-shaped red blood cells to their standard shape at a concentration of 50 µL/mL. A hypoxia-induced hemolysis assay was performed (Ngbolua *et al.* 2016), basically the erythrocytes were centrifuged and resuspended with phosphate buffer and sodium metabisulfite, incubated at 37ºC for 60 minutes, with and without the extract of organic acids. Removing aliquots at certain times and centrifuged, thus determining the absorbance versus time. This test showed inhibition of hemolysis of 57%. The osmotic fragility test also evaluated red cell fragility. Blood was added in series in a hypotonic saline solution with different concentrations. After the organic acid extract was added, the results of the number of red blood cells lysed by saline concentration were performed by a photonic microscope and a

hemocytometer. It was shown that with increasing saline concentration, there was a decrease in hemolysis, but the lysis rate of treated red cells was greater than that of the control (sick red cells alone). The ITANO assay, hemolysis of Sickle red blood cells (RBCs) in isotonic conditions, the haemoglobin (Hb) S polymerization was performed. The formation of met-haemoglobin was also evaluated, a derivative of haemoglobin with heme iron in a ferric state, which cannot supply oxygen, occurring in a state of hypoxia (Kaewprayoon *et al.* 2020). They resulted in a significant decrease in met-haemoglobin formation.

All four studies (Amoo *et al.* 2012; Fernandez *et al.* 2017; Gazim *et al.* 2014; Sabela *et al.* 2017) showed antioxidant activity, with the exception of fraction 16 (9β,13β-epoxy-7-abietene) reported by Gazim *et al.* (2014). Three of them were made from the plant leaf extract (Amoo *et al*. 2012; Fernandez *et al.* 2017, Sabela *et al*. 2017). Two of the four used some fraction (Fernandez *et al.* 2017; Gazim *et al.* 2014) and one used TrEO (Gazim *et al.* 2014)

Amoo *et al.* (2012) analysed 21 medicinal plants, including *T. riparia*. Comparisons were made between fresh and stored plant material (for *T. riparia*, 16 years). After the plant material was transformed into a fine powder and mixed with 50% methanol, the extraction was carried out. In the methods designed to test the antioxidant potential, assays with DPPH (2,2-diphenyl-1-picrylhydrazyl, DPPH) were used (Fawole *et al.* 2010; Amoo *et al.* 2012). The plant extract mixed in DPPH solution and methanol was a negative control for the positive ascorbic acid and butylated hydroxytoluene (BHT). The absorbances were measured. Equations for radical scavenging activity (RSA) were used to find the Antioxidant Activity Index (Scherer and Godoy 2009; Amoo *et al.* 2012). For RSA, the results of 68.5% and 23.8% were obtained for the stored and fresh plants, respectively. The antioxidant activity index obtained for the fresh material was not determined, it was 0.5. for the stored material.

A b-carotene / linoleic acid model system was also performed, where the coupled inhibition of b-carotene and linoleic acid oxidation was measured (Moyo *et al.* 2010; Amoo *et al.* 2012). The plant extract was evaluated at a final concentration of 200 µg/mL. Absorbances were measured every 30 minutes for 2 hours. For this method, the percentage of antioxidant activity was discovered by an equation, where they obtained the result of 64.5% for fresh material and 67.2% for stored material (Amoo *et al.* 2012). Fernandez *et al.* (2017) performed the same model but described by Mattos *et al.* (2009) and, with some modifications, obtained the result for the crude extract (fresh material) of 51.52% inhibition of oxidation. Following the fact that they also tested

the inhibition for some fractions, they obtained the following results FR-I 80.15%, FR-II 39.57%, FR-III 15.76% and FR-IV 55.61%. Gazim *et al.* 2014 performed this assay described by Kumaran and Karunakaran (2006), and Fraction 17 showed high antioxidant activity, requiring concentration (109.6 μg mL^{-1}) lower than the positive control (BHT) to inhibit 50% of the oxidative activity. TrEO also showed better activity than BHT, being 130.0 μg/mL and 0133.5 μg/mL, respectively. Fraction 16 did not show antioxidant activity for this assay.

In the study by Sabela *et al.* (2017), they used the plant extract nanocarrier in silver nanoparticles (AgNPs). They performed the extraction of diterpenes from the plant leaves with water and incorporated them into the rest of the materials to synthesize nanoparticles. The antioxidant outcomes were performed by eliminating free radicals from the solution of 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) by the nanoparticles. The biosynthesized AgNPs were added to the ABTS solution and mixed by vortexing and incubated for 30 minutes, and the absorbance was read. They showed a percentage of free radical scavenging efficiency of 96.62% at 0.20 mL/0.05 mM, showing excellent antioxidant activity (Sabela *et al.* 2017). For another article, we performed this trial (Gazim *et al.* 2014) using the method described by Ozgen *et al.* (2006) to determine the ability of TrEO and fractions 16 and 17 to scavenge radicals from the ABTS solution. The result of this study was expressed in the concentration at which both TrEO and the fractions took to eliminate 50% of free radicals. Fraction 16 did not show antioxidant capacity. Both EO (1524 μg/mL) and fraction 17(1024 μg/mL) presented a higher concentration than the positive control (190 μg/mL), therefore, a lower potential in this test.

Other authors used the crude extract and some fractions of greater interest, such as FR-I, FR-II, FR-III and FR-IV (Fernandez *et al.* 2017) (Table 1). They used four methods to assess the antioxidant potential: the quantification of total phenols, DPPH free radical scavenging, the beta-carotene/linoleic acid system method (already mentioned above) and the iron reduction/antioxidant power (FRAP) method (Table 2). In the first method (Singleton and Rossi 1965; Viuda-Martos *et al*. 2010; Fernandez *et al.* 2017), the samples were diluted in methanol to a concentration of 1.0 mg/mL, after which an aliquot of each sample was mixed in the Folin– reagent. Ciocalteau and sodium carbonate and kept in a bain-marie. Absorbance reading was performed, the concentration of total phenols was calculated from the standard curve of gallic acid, being expressed by its concentration in the sample. The FR-IV showed the best result,

which was 181.97 µg of gallic acid/mg of sample, followed by FR-II (119.85 µg/mg), crude extract (94.31 µg/mg), FR-III (76.94 µg/mg) and finally FR-I (34.27 µg/mg).

The DPPH test performed by Fernandez *et al.* (2017) basically was the same trial already mentioned here (Amoo *et al*. 2012). They used the extract concentrations of fractions of 1.0, 0.75, 0.5, 0.25, 0.125 and 0.0625 mg/mL and mixed in the methanolic solution of DPPH. The results were expressed by the concentration of antioxidant samples required to reduce free radicals by 50% (EC50). FR-IV decreased 50% of free radicals with a lower concentration (0.62 mg/mL) of extract. The results obtained for the other samples were 0.91 mg/mL, 10.41 mg/mL, 0.88 mg/mL and 1.75 mg/mL for the crude extract, FR-I, FR-II and FR-III, respectively. Gazim *et al.* (2014) tested EO's free radical scavenging potential and fractions 16 and 17 (Mourão *et al.* 2011, Molyneux 2004). Fraction 16 again did not show activity for this test. Fraction 17 had the lowest concentration (0.010 µg/mL) to reduce 50% of free radicals, better than Quercetin (positive control) which was 2.05 µg/mL, and for TrEO, the concentration was 15.63 µg/mL.

In the study by Fernandez *et al.* (2017), the FRAP method was also performed (Benzie and Strain 1996; Rufino *et al.* 2006 and Fernandez *et al.* 2017), basically in each sample with different concentrations (500; 250; 125 and 62.5 µg/mL) water was added and the FRAP reagent, thoroughly homogenized and left to rest for 30 minutes at 37ºC. Absorbances and a standard curve for ferrous sulfate were read. FR-IV again showed an antioxidant activity (4.59 µM/mg) expressed by µM of ferrous sulfate/mg of sample. FR-I had the worst result for antioxidant activity (0.34 µM/mg). The other results were 2.04 µM/mg, 2.23 µM/mg and 1.89 µM/mg for the crude extract, FR-II and FR-III, respectively.

3.2.2 Antimicrobial activity

The antimicrobial activity of *T. riparia* was reported *in vitro* by 18 studies (Baldin *et al.* 2018; Cardoso *et al*. 2018; Demarchi *et al*. 2015a and 2016; Endo *et al.* 2015; Fernandez *et al.* 2017; Gazim *et al.* 2010*;* Kakande *et al*. 2019; Melo *et al.* 2015a and 2015b; Ndiku and Ngule 2014; Ngbolua *et al.* 2016; Ngule *et al.* 2014; Okem, Finnie and Van Staden, 2012; Terron-Monich *et al.* 2018; Van Puyvelde *et al*. 1986; Van Puyvelde *et al.* 2018 and York, Van Vuuren and Wet, 2012). Only one study was tested *in vivo* (Cardoso *et al*. 2018) (Table 1).

A total of ten studied bacterial microorganisms (Baldin *et al.* 2018; Fernandez *et al.* 2017; Gazim *et al.* 2010; Melo *et al.* 2015a; Ndiku and Ngule 2014; Ngbolua *et al.* 2016; Ngule *et al.* 2014; Okem, Finnie and Van Staden, 2012; Van Puyvelde *et al.* 1986; York, Van Vuuren and Wet, 2012), six antifungal activity (Endo *et al.* 2015; Gazim *et al.* 2010; Kakande *et al.* 2019; Okem, Finnie and Van Staden, 2012; Van Puyvelde *et al.* 1986; York, Van Vuuren and Wet, 2012), four observed actions on protozoa (*Leishmania*) (Cardoso *et al.* 2018; Demarchi *et al*. 2015a and 2016; Terron-Monich *et al.* 2018), and three anthelmintic activity (Melo *et al.* 2015b; Okem, Finnie and Van Staden, 2012; Van Puyvelde *et al.* 2018) (Table 1 and 2).

Bacterial

Of the 24 studies, 10 performed tests to determine the plant's antibacterial activity (Baldin *et al*. 2018; Fernandez *et al.* 2017; Gazim *et al.* 2010; Melo *et al.* 2015a; Ndiku and Ngule 2014; Ngbolua *et al.* 2016; Ngule *et al.* 2014; Okem, Finnie and Van Staden, 2012; Van Puyvelde *et al.* 1986; York, Van Vuuren and Wet, 2012) (Table 2), all of which are *in vitro* tests (Table 1).

All studies used the leaves, with six treating the extract's potential (Fernandez *et al.* 2017; Ndiku and Ngule 2014; Ngbolua *et al.* 2016; Ngule *et al.* 2014; Okem, Finnie and Van Staden, 2012 and York, Van Vuuren and Wet, 2012), three TrEO (Baldin *et al.* 2018; Gazim *et al.* 2010 and Melo *et al.* 2015a;), one TrROY (Baldin *et al.* 2018), and one a single plant isolated (Van Puyvelde *et al.* 1986) and 1 dealt with fractions (Fernandez *et al.* 2017) (Table 1).

The variety of bacterial genera studied was diverse among the articles. Ten studies showed activity on some bacteria, With emphasis mainly on gram-positive bacteria. Fernandez *et al.* (2017) evaluated the Minimal inhibitory concentration (MIC) of the crude extract (CE) and some fractions, previously mentioned, at a concentration of 0.49 to 500 µg/mL using the microplate microdilution method with 96 wells in U-bottom, in Mueller Hinton broth, adjusted with calcium and magnesium cations (CAMBH). The results were interpreted by the lower concentration of CE and fractions with no colour, which means that they can inhibit bacterial growth (Berridge *et al.* 1996; Fernandez *et al.* 2017). FR-I (abieta-7,9(11)-dien-13-beta-ol) required a lower concentration to inhibit the growth of *Staphylococcus aureus* (0.9 8µg/mL), *Enterococcus faecalis* (31.2 µg/mL) and *Bacillus cereus* (31.2 µg/mL) and for *Escherichia coli* and *Salmonella Typhimurium*, 125 µg/mL and 62.5 µg/mL, respectively. FR-II also showed good results for MIC, especially for *B. cereus* (62.5

µg/mL) (Table 2). They did not find a great inhibition potential for *Pseudomonas aeruginosa* and *Aeromonas hydrophila* in any of the fractions or the CE.

Van Puyvelde *et al.* (1986) isolated one of the FR-III compounds (Fernandez *et al.* 2017), 8(14),15-sandaracopimaradiene-7alpha,18diol, extracted with chloroform (Table 1). They used the liquid dilution method, with diterpenediol concentrations 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 and 0.39 µg/mL. Getting better inhibition of gram-positive bacteria. Compared to the previous study (Fernandez *et al.* 2017), *S. aureus* required a 500 µg/mL concentration, the FR-III isolate showed greater inhibition, with a MIC of 12.5 µg/mL (Van Puyvelde *et al.* 1986). For gram-negative bacteria, it showed significant values for *P. vulgaris* (25 µg/mL), *P. solanacearum* (25 µg/mL), and *Shigella dysenteria*e (12.5 µg/mL) (Table 2).

Ngbolua *et al.* (2016) using the same method, tested MIC and the minimal bactericidal concentration (MBC) (trypticase soy agar, TSA) for *S. aureus* and *E. coli*. They obtained the results for *E. coli* of 31.25 µg/mL of the organic extract, this result refers to the MIC and MBC value. As for *S. aureus*, the extract had a result of 125 µg/mL.

Okem, Finnie and Van Staden (2012) described the difference in the antibacterial potential of *T.riparia* extracts obtained from different solvents (ethyl acetate, ethanol and water) for *E. coli, S. aureus* and *E. faecalis.* It was considered to be a good antibacterial agent with results less than 1 mg/mL (Table 1). The extraction performed with Ethyl acetate showed better results for *E. coli* and *E. faecalis*, MIC of 0.19 and 0.04 mg/mL. As for *S. aureus*, the ethanol extract showed the best result at 0.39 mg/mL. The results for the other solvents presented values lower than 1 mg/mL. The results for MBC were not significant (Table 2).

Ngule *et al.* (2014) used water as an extracting solvent, tested in different bacteria cultures, on Mueller Hinton agar (Table 1). All showed a zone of inhibition, with *E.coli* having the slightest halo (9.67 mm). As for *Salmonella typhi*, the extract showed a large inhibition halo, very close to the positive control, 21.00 mm and 22.67 mm, respectively. Followed by *S. epidermidis*, *B. cereus* and *P. vulgaris*, all values around 18 mm. The two studied species of the genus *Serratia*, on the other hand, presented an inhibition halo of 13 mm (Table 2).

Ndiku and Ngule (2014) performed an infusion of the plant's leaves to analyze the effects of the extract. Strains of *Serratia liquefacien*s, *Salmonella typhi*, *Proteus vulgaris*, *E. coli*, *Enterobacter aerogenes* and *B. cereus* were tested (Table 1). The plant extract could inhibit all species tested, emphasising *B. cereus* with a 22.67 mm

inhibition halo, followed by *S. liquefaciens* with a 21.00 mm halo. Compared to the previous study (Ngule *et al.* 2014), *E. coli* presented a slightly larger halo in this study of 13.33 mm, this same value for *S. typhi*, much smaller than the previous study (21.00 mm) (Ngule *et al.* 2014). Other results for tested microorganisms were*: E. aerogenes* 14.33 mm and *P. vulgaris* 12.33 mm (Table 2).

York, Van Vuuren and Wet studied some bacteria causing respiratory infections (2012). From the extraction of *T. riparia* leaves with dichloromethane-methanol and another with water, microdilution was performed to express the MIC values, when < 1 mg/mL were considered notorious values (Table 1). The organic extract showed the lowest concentrations necessary to inhibit infectious agents. The best result for *S. aureus* was 0.03 mg/mL, followed by *Moraxella catarrhalis* 0.10 mg/mL (Table 2).

TrEO was also tested as an antibacterial agent by Gazim *et al.* (2010), who tested extracts of different seasonality against eight other bacterial species (Table 1). In the diffusion disk method, *K. pneumoniae* showed the most significant inhibition in autumn (24.3 mm), greater until the positive control used, Genthamicine (20.6 mm) followed by *S. aureus*, which in the oil extracted from the leaves in the summer showed inhibition of 23.3 mm. Like *E. coli, S. enterica* and *B. cereus* also showed better inhibition with leaves collected in the summer. *E. faecalis* and *P. aeruginosa*, the best values for autumn, and in the latter, the value was the same for winter. As for *P. mirabilis*, it did not show a halo of inhibition for all OE. The results of MIC *S. aureus* and *B. subtilis* presented the lowest inhibitory values, in concentrations ranging from 7.80 to 31.2 µg/mL, with emphasis on TrEO performed in the summer, with a MIC value of 7.80 µg/mL. In summary, the microorganisms that showed more significant variations concerning the year's season were *S. aureus* and *B. subtilis* gram-positive bacteria. York, Van Vuuren and Wet (2012) used TrEO, in their study considered notorious inhibition values ≤ 2.00 mg/mL, and for *S. aureus*, *M. smegmatis*, *M. catarrhalis* and *K. pneumoniae* they were above this value (Table 2).

Microdilution using CAMBH was also a method to assess the effects of TrROY isolated from TrEO (Baldin *et al.* 2018) (Table 1). The results for this compound were better for gram-positive bacteria, such as *E. faecalis* and *S. aureus*, both with a MIC of 62.5 µg/mL (Table 2). Finally, Baldin *et al.* (2018) tested, in addition to TrROY, the effects of EO on the bacterium that causes tuberculosis, *Mycobacterium tuberculosis*. For the test, 19 clinical isolates were used (7 being susceptible to anti-tuberculosis drugs, 6 to isoniazid and 6 multi-resistant to drugs), and one strain was also studied. The Resazurin Microtiter Assay Plate (REMA) method was used (Palomino *et al.* 2002,

Baldin *et al.* 2018). Both EO and TrROY were diluted to a concentration of 0.98 to 250 µg/mL and added to microplates together with the inocula of the microorganism. After the seven day incubation, resazurin solution was added. The interpretation was made by changing the colour from blue to pink, which indicates that there is bacterial growth. TrROY proved to be a compound with a potential agent against *M. tuberculosis*, considering that the MIC results obtained for all isolates were 31.2 µg/mL. For TrEO, the values varied between 31.2 and 62.5 µg/mL. According to the study by Gu *et al.* (2004), good candidates for a drug for the management of tuberculosis have an inhibitory concentration of 64 µg/mL (Table 2).

Fungal

The antifungal activity were investigated by six studies (Endo *et al.* 2015; Gazim *et al*. 2010; Kakande *et al*. 2019; Okem, Finnie and Van Staden, 2012; Van Puyvelde *et al.* 1986; York, Van Vuuren and Wet, 2012) (Table1). All tested the leaves from the plant. Three studies tested on population dermatophyte fungi that encompass the genera *Trichophyton, Microsporum* e *Epidermophyton* (Endo *et al.* 2015; Kakande *et al*. 2019; Van Puyvelde *et al.* 1986). Three studied *Candida albicans* (Gazim *et al*. 2010; Okem *et al*. 2012; Van Puyvelde *et al.* 1986), and one *Cryptococcus neoformans* (York, Van Vuuren and Wet, 2012) (Table 1).

Of the six studies dealing with the plant's antifungal potential, five showed activity (Endo *et al.* 2015; Gazim *et al.* 2010; Kakande *et al.* 2019; Okem, Finnie and Van Staden, 2012; and York, Van Vuuren and Wet, 2012). In 1 of them, it did not show good activity against fungi (Van Puyvelde *et al.* 1986)

In their study Endo *et al*. (2015) used the hydroalcoholic extract from the leaves of the *R. officinalis, P. guajava* and *T. riparia* plants. The 96-well plate microdilution method estimated the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC), which for the plant also known as false myrrh was 62.5 to 250 mg/mL (Table 1). The disk-diffusion method was also performed, where *T. riparia* presented satisfactory results for the three microorganisms (Endo *et al*. 2015). In addition to the disk diffusion method, a fluorescence microscope was used to measure the inhibitory concentration of hyphae growth, where it confirmed the result obtained in the disk-diffusion method, which was no different when analyzed in the scanning electron microscope. This study showed that the extract had more inhibitory action for *T. rubrum* and *T. mentagrophytes* than for *M. gypseum* (Table 2). Kakande *et al.* (2019)

applied the same method, and tested the species *T. tonsurans*, *T. mentagrophyte*, and *M. audouinii.* All were susceptible to the crude extract at a concentration of 1 g/mL(Table 1), *T. tonsurans* was more susceptible, presenting a MIC of 62.5 mg/mL, followed by *T. mentagrophyte* (125 mg/mL) and finally *M. audouinii* (250 mg/mL). It was obtained MFC with low values for *T. tonsurans* and *T. mentagrophyte* suggesting that both were susceptible to the extract, while for *M. audouinii* it showed high MFC value, suggesting that this species of dermatophyte was less susceptible to the extract of *T. riparia*. (Table 2)

Van Puyvelde *et al.* (1986) isolated a diterpenediol (8(14),15-Sandaracopimaradiene-7Q8diol) in chloroform solvent. Serial dilutions were used for antimicrobial determination (Table 1), to the concentration at which there was no further growth. The result of this study for antifungal action was not as efficient as for antibacterial (Table 2).

The variations, in relation to the seasons, of the compounds present in the plant were analyzed, according to Gazim *et. al* (2010) there is really a seasonal difference in the composition of the TrEO. *Candida albicans*, showed to be very sensitive to EO, in all periods, tested through the microdilution method and calculated the MIC and also by disk-diffusion (Table 1). The zone of inhibition for this microorganism was 19.3 to 22.3 mm and the MIC was 31.2 to 62.4 µg / mL, the best activity was presented in the summer. The extraction methods used by Okem, Finnie and Van Staden (2012), have already been reported in this study, their results show that the extracts are pharmacologically active against *Candida albicans*. After using the same method as Gazim *et al*. (2010), obtained results from MIC 0.39 to 12.5 mg/mL and MFC 1.56 to 6.25 mg/mL, with ethyl acetate being the best solvent (Table 2).

The investigation of the use of plants in the treatment of infectious diseases in the respiratory tract was the objective of one of the studies (York, Van Vuuren and Wet, 2012). And it presented the activity of 30 species of plants, in some bacteria, as already mentioned, but also for the fungus of the species *Cryptococcus neoformans.* The method used was based on microdilutions of the extract of a single plant or mixtures of more than one species. Two types of organic and aqueous extracts were obtained, for which the results for Iboza riparia were 0.60 and 1.00 mg/mL. For TrEO the MIC presented was 0.83 mg/mL.

Leishmaniasis **and other protozoa**

Of the 24 articles, four of them dealt with the pharmacological potential of TrEO on the protozoan of the genus *Leishmania* (Cardoso *et al.* 2018; Demarchi *et al.* 2015a and 2016; Terron-Monich *et al.* 2018), and two also studied the effect of the 6,7-dehydroroyleanone (TrROY) isolated from EO (Demarchi *et al.* 2015a; Terron-Monich *et al.* 2018) (Table 1). In two studies, tests were conducted for the promastigote and amastigote (intracellular) forms (Cardoso *et al.* 2018; Demarchi *et al.* 2015a), while the other two only for the intracellular form (Demarchi *et al.* 2016; Terron-Monich *et al.* 2018) from *Leishmania (Leishmania) amazonensis* (Table 1). All four studies performed in vitro experiments (Cardoso *et al.* 2018; Demarchi *et al.* 2015a and 2016; Terron-Monich *et al.* 2018). Only one of the authors conducted an in vivo assay of *anti-Leishmania* activity in BALB/c mice infected (Cardoso *et al.* 2018) (Table 1).

All four studies showed significant effects of TrEO against *L. (L.) amazonensis* (Cardoso *et al.* 2018; Demarchi *et al.* 2015a and 2016) and TrROY (Terron-Monich *et al.* 2019) (Table 2). TrEO was more potent *in vitro* against promastigote and amastigote forms than its isolate (Cardoso *et al.* 2018), it was also observed by Demarchi *et al*. (2015a) (Table 2).

Only one study evaluated the in vivo activity (Cardoso *et al.* 2018) in BALB/c infected female rats, using topical TrEO, extracted in the summer, at 0.5% and 1% concentrations (Table 1). After evaluating the topical test, the parasite load in the lymph nodes and spleen of mice was quantified following the method described by Lonardoni *et al.* (2000), by microdilution in 96-well plates and counting the infected cells under a microscope. This experiment showed no reduction in the skin lesion, but the parasite load decreased in the spleen. The study carried out to assess in vitro anti-*Leishmania* activity by Cardoso *et al*. (2018) showed an excellent inhibitory potential on the growth of promastigote forms, with IC50 ranging from 13.31 to 15.67 ng/mL, better than the positive control. Cardoso *et al.* (2018) observed that the topical administration was not effective to cure the skin lesions at the concentration studied (Cardoso *et al.* 2018) (Table 2).

Demarchi *et al.* (2016) were performed from macrophages infected with *L. (L.) amazonensis* promastigotes and treated with TrEO 30 ng/mL (Table 1). Microscopic counting of infected amastigotes and macrophages was performed under an optical microscope. At the concentration used, there was a 50% decrease in the infection rate. The effects of TrEO on the parasite load in infected macrophages was also verified by the qPCR method (Table 1), with a 91% decrease in those macrophages treated with TrEO (Table 2).

TrEO and the TrROY were tested against promastigote and amastigote of *L. (L) amazonensis* (Demarchi *et al.* 2015a). For the promastigote forms, parasite viability was first evaluated by the XTT method and defined by the dose that reduced 50% (LD50) of the survival of treated parasites compared to untreated ones (Table 1). The TrEO showed better activity (LD50= 0.5 µg/mL), with no significant difference for AmB, the reference drug. TrROY showed less effectiveness as it required a higher concentration to reduce parasite survival. Microscopic counting showed that the concentration needed to inhibit growth by 50% (IC50) was 0.03 µg/mL for TrEO, while for the isolate, it was 2.45 µg/mL. The changes that occurred in the parasite structures were analyzed by a transmission electron microscope at a concentration of 0.03 µg/mL and 0.5 µg/mL of TrEO. In both concentrations, there were changes in the morphology of the promastigote form of the parasite (Table 2).

The intracellular effects of TrROY on amastigotes was tested by Terron-Monich *et al.* (2018). Briefly, murine macrophages infected with *L. (L.) amazonensis* were treated with the isolate (Table 1). The percentage of infected cells was multiplied by the mean number of parasites per cell, the rate of murine macrophage infection reduction was 31% at a concentration of 0.1 µg/mL (Terron-Monich *et al.* 2018) (Table 2). This same test was performed by Demarchi *et al.* (2015a) and showed a very similar reduction found by Terron-Monich *et al.* (2018). At a concentration of 0.03 µg/mL, it did not show cytotoxicity and there was a 65% reduction in infection (Demarchi *et al.* 2015a) (Table 2).

Helminths

Of all the studies, only three dealt with the anthelmintic activity of the plant (Melo *et al*. 2015b; Okem, Finnie and Van Staden, 2012; Van Puyvelde *et al.* 2018), within them two used *Caenorhabditis elegans* as a population (Okem, Finnie and Van Staden, 2012; Van Puyvelde *et al*. 2018) and the other *Schistosoma mansoni* (Melo *et al.* 2015b). All tests were performed in vitro and were obtained through the leaves of the plant, but with different derivatives. (Table 1 and 2). All 3 achieved positive results in their anthelmintic action.

It was used by Melo *et al.* (2015b) the essential oil obtained by the hydrodistillation method in a Clevenger-type apparatus. For the Schistocimidal assay, dilution of TrEO in DMSO at different concentrations was used and then the MTT method was followed (Magalhães *et al.* 2012; Melo *et al*. 2015b) to assess the viability

of the parasites (Table 1). It presented positive results since there was a decrease in the viability of the worms after treatment with the lowest concentration of TrEO (10 µg/mL) and at the highest concentration (100 µg / mL) similar to the control group (Table 2).

In another of the articles, extraction was performed with ethyl acetate, ethanol and water from the leaves of the plant. To determine the viability of *C. elegans*, microdilution tests were used for each plant extract. Extracts of different plants were tested (*Canthium spinosum, Cassinopsis ilicifolia, Coddia rudis, Conostomium natalensis, Crassula multicava, Lagynia lasiantha and T. riparia*) and the extract of *T. riparia* with ethyl acetate stood out (Table 1). It was showing the best activity anthelmintic with a minimum lethal concentration (MLC) of 0.004 mg/mL, given that the study found that plant extracts with an MLC value of less than 1 mg/mL had high anthelmintic activity (Okem, Finnie and Van Staden, 2012) (Table 2).

In their study, Van Puyvelde *et al.* (2018) investigated the pharmacological potential of the active ingredient 8(14),15-sandaracopimaradiene-7α,18-diol extracted with the solvents hexane, ethyl acetate, methanol and water (Table 1). The assay was performed in a 96-well microplate, added together with the larvae, plant extract, the fraction or stock solution of the pure compound and the result was estimated by the inhibitory concentration (IC50). Among the findings presented, it was concluded that only the hexane extract was active, after which the active principle was isolated, with anthelmintic potential 8(14), 15-sandaracopimaradiene-7α,18-diol, with an IC50 of 5.4 ± 0.9 µg/ml (Table 2).

3.2.3 Anti Inflammatory and immunomodulatory actions

Of the 24 studies, a total of three performed in vitro immunomodulatory assays, two tested TrEO (Demarchi *et al.* 2015b and 2016) and one TrROY (Terron-Monich *et al*. 2018) (Table 1). All studies showed that both TrEO and TrROY produced modulatory effects on the studied murine macrophages (Table 2).

In studies conducted by Demarchi *et al.* (2015b and 2016), murine peritoneal macrophages obtained from BABL/c mice were exposed to 30 µg/mL of TrEO. After 3, 6 and 24 hours, the supernatants were analyzed in a flow cytometer for cytokine determination and a semi-quantitative RT-PCR reaction for cytokine mRNA expression was also performed (Table 1). mRNA expression and cytokine production occurred at a low concentration of TrEO. EO induced the expression of interleukins (IL) 1β, IL-2, IL-

12, IL-17 and interferon-γ. Granulocyte and monocyte colony-stimulating factors and IL-17 were produced at high levels. And they decreased the regulation of IL-10 and IL-6. It is worth remembering that the effects varied with the incubation time concerning the expression of pro-inflammatory cytokines in macrophages, none of which was expressed after 24 hours (Table 2).

Demarchi *et al.* also investigated the effects of TrEO on murine macrophages infected with *L. (L.) amazonensis* and treated with 30 µg/mL TrEO (Table 1). The findings found that TrEO inhibited some important cytokines for the parasite's growth, such as a stimulating factor for granulocyte and macrophage colonies, IL-4, IL-10 and TNF. TrEO inhibited the blockade of IFN-gamma and IL-12 promoted by the parasite (Table 2).

Terron-Monich *et al.* (2018) also studied the immunomodulatory effects of TrROY on murine macrophages infected and non-infected by *Leishmania* (Table 1). Based on previous studies (Demarchi *et al.* 2015b and 2016), and with the same study population (Table 1), macrophages were exposed to concentrations of 0.1, 1 and 100 µg/mL of TrROY. Cytokines levels were determined by flow cytometry (Table 1). The result was that TrROY did not show significant activity on IL-1β, GM-CSF, IL-2, IL-5, IL-10 or TFN-α. TrROY enabled the increase of IL-12 and the decrease of IL-4 at a concentration of 0.1µg / mL, which does not show cytotoxicity (Table 2).

The anti-inflammatory effects were also observed in cyclooxygenase (COX) inhibitory activities (Oken, Finnie and Van Staden 2011) (Table 1). They used the plant leaf extract extracted with ethyl acetate, ethanol and water. Organic extracts were used at a concentration of 250 µg/mL and aqueous 2 mg/mL. After analyzing the amount of radioactivity present in the sample compared to the blank and expressed as a percentage of COX inhibition. Leaf extraction with ethyl acetate proved to be more inhibitory for COX-1 and COX-2 enzymes (Table 2). Ethanol extract showed inhibitory activity for COX-2, while aqueous extract showed excellent inhibition activity for the COX-1 enzyme, around 70% (Table 2).

Gazim *et al.* (2010) tested the antinociceptive activity in vivo in Swiss albino mice, performed by acetic acid-induced abdominal constriction. One hour before the stimulus, a suspension with EO was administered orally, at a dose of 200 mg/kg and Indomethacin 10 mg/kg as a positive control (Table 1). And the number of constrictions was measured over 20 minutes. It inhibited pain activity in percentages from 38.94% to 46.13%. In this study, they performed the variation of the EO seasonally, and for this test, there were no significant differences between the seasons of the year (Table 2).

3.2.4 Other potential actions

Acetylcholinesterase inhibitory activity

Only one study conducted an assay on the action of plants on the neurotransmitter Acetylcholine (ACh) (Amoo *et al.* 2012) (Table 1). An in vitro test was performed using the colorimetric method (Eldeen, Elgorashi and Van Staden, 2005; Amoo *et al.* 2012) comparing with extracts from fresh leaves and with plant material stored for 16 years. As a positive control, they used Galantamine, a tertiary alkaloid, a selective, competitive and reversible inhibitor of acetylcholinesterase (AChE) (Anvisa, 2021), commonly used in Alzheimer's disease. The percentage of acetylcholinesterase inhibition was calculated (Table 1). A number of plants, both fresh and stored for 12 or 16 years, were tested. *T. riparia*, at a concentration of 1.0 mg/mL, presented one of the best potentials for AChE inhibition. For fresh material it was 65.4% inhibition and 80.8% for stored material, concluding then that the stored material presented a greater inhibition, but that both presented an AChE inhibitory activity (Amoo *et al.* 2012) (Table 2).

Acaricidal and larval activity

TrEO's acaricidal activity was evaluated in only one of the studies (Gazim *et al.* 2011) (Table 1). *Rhipicephalus (Boophilus) microplus* was used as the population studied. It is a very valuable study, considering that at the moment chemical agents are used that cause resistance to the active principle. (Chagas *et al*. 2003; Gazim *et al.* 2011)(Table 2). Both for the acaricidal test (AIT) and for the larval packet test (LPT) a serial dilution of the TrEO was used, adding an emulsifying agent (Tween 80). A group of pregnant females was added, removed and incubated for 14 days. Afterwards, it was quantified to females that laid eggs. These eggs were weighed and incubated for 21 days and the efficacy of treatment against engorged females was assessed by measuring mortality of gravid females, total number of eggs, egg weight, percentage of hatchability, and product efficiency (Gazim *et al.* 2011) (Table 1). For LPT larvaes were wrapped in filter-paper envelopes containing solution tests and incubated for 24 hours, this test was measured by percent mortality of *R. (B.) microplus* larvae exposed to different concentrations (Table1). The results obtained were that mortality is dose-dependent, in lower concentrations the rate of mortality was high, for example in a

concentration 12,50%(w/v), the average percentage was 97,60%. This result is so promising for animals and humans health (Table 2).

Repellency activity

The plant's repellent activity was tested in humans in one of the studies, on the *Anopheles gambiae* mosquito of principal malaria vector (Omolo *et al.* 2004) (Table 1). Six humans were selected for the test, where none showed allergic or mild reactions, both to bites and to EO (Table 2).

Briefly, the method was based on the use of 25 hungry females of *An. gambiae*, trapped in a cage, one of the arms that were used as a control (dispensed with acetone) was inserted for 3 minutes and the counting of how many mosquitoes were performed. they landed on his arm. Then, the treated arm was inserted from the lowest to the highest concentration ($10^{-5}, 10^{-3}$ and 10^{-1}), for the same period of time and the number of females that landed was recorded. The 6 replicates were expressed as protective efficacy, which consists of the % of the control mean subtracted from the % of the test mean, divided by the % of the control mean (Mehr *et al.* 1985; Omolo *et al*. 2004). TrEO had repellent activity comparable to synthetic repellents (Omolo *et al.* 2004) (Table 2).

4. DISCUSSION

In this review, Brazil was the country with more publications on the *T. riparia* pharmacological potential. The other regions were also located in tropical areas. As mentioned earlier, this plant is grown in areas of subtropical and temperate climate and is found in Brazil, Africa and Asia as an ornamental plant in gardens, parks, houses, and mainly, due to its intense aroma. Due to its pharmacological properties, it is used in the treatment of infectious and inflammatory diseases in traditional African and Asian medicine. *T. riparia* is also known as *Iboza riparia* and *Moschosma riparium* (Martins *et al.* 2008). In South Africa, it is one of the most popular herbs and medicinal plants (Van Puyvelde e De Kimpe, 1998).

Most studies used leaves to obtain an extract, essential oils and isolated compounds. This is because compounds with pharmacological potential are found in the leaves of plants, mainly because the *T. riparia* plant is a shrub, with leaves being its main resource for obtaining drugs (Gazim *et al.* 2010).

There has been a significant increase towards more natural alternatives for treating certain conditions. Among them, an old traditional treatment is the use of EO. There are reports that Hippocrates, the father of medicine, used aromatic fragrances to cure illnesses (Lee *et al.* 2019). The term "essential oil" is called "regular product from vegetable raw material, either by distillation with water or steam, or from citrus fruit epicarp by a mechanical process, or by dry distillation" (ISO 9235, 1997). The use of essential oils in different areas, such as pharmaceuticals, food and cosmetics, is becoming more popular every day (Turek and Stintzing 2013). They are a mixture of compounds in ideal proportions, acting synergistically for the effect of the plant. (Scuteri *et al.* 2021). OEs, in general, are lipophilic substances with high volatility, a complex mixture of components, the vast majority of which are lipophilic terpenoids, derivatives of aliphatic chain hydrocarbons, and reducing phenylpropanoids, with terpenoids being the most frequent (Kubeczka 1979; Turek and Stintzing 2013).

Many of the studies brought the use of EO from the *T. riparia* plant, when performing the chemical composition analysis by gas chromatography coupled with mass spectrometry (GC-MS) it was described to be a complex rich in terpenoids (Gazim *et al.* 2010). The EO was extracted mainly from the Clevenger method and the granted or fractions by gas chromatography. Even so, the yield of the plant to obtain the essential oil was given by the leaves, as it is the main material of the plant, the yield value was 0.17% to 0.26% (Gazim *et al.* 2010). This variation occurs due to the season in which the plant material was collected to obtain de EO. Gazim *et al.* (2010) and Cardoso *et al.* (2018) reported that the maximum value occurring in winter and the minimum in spring, the factors that occurred in this variation are diverse, and may be due to the amount of rain, humidity, light and temperature (Sarma 2002; Cardoso *et al.* 2018). Changes only occurred in the requirements of the compounds and not in their chemical composition. The major compounds in all seasons of the year were oxygenated sesquiterpenes. (Gazim *et al*. 2010; Cardoso *et al.* 2018).

TrROY, a diterpene isolated from EO, was also analyzed for its effects, but often compounds isolated from EOs may not have the same effect, requiring higher concentrations to achieve the desired effect, (Bassole and Juliani, 2012; Demarchi *et al*. 2015a). In comparison, TrEO is more cytotoxic to host cells than TrROY. This was expected since TrEO is a complex mixture of terpenoids (Baldin *et al*. 2018). However, the fact of increasing the concentration can generate a toxic effect.

Most of the studies included in this review performed in vitro tests and different types of the cell population (BALB/c mouse murine macrophages, human and lineage

tumour cells). According to Demarchi *et al*. 2015a and b, TrEO showed more significant cytotoxic effects to murine macrophages than TrROY, most likely because the oil is a rich complex of interacting substances. Also, according to Demarchi *et al*. (2015a) and Gazim *et al*. (2014), TrEO did not show toxicity in human cells. The results also corroborate the findings of Baldin *et al.* (2018)

Although, in most drug treatments, low toxicity is sought, in some cases, the cytotoxic effect can be of value for the treatment of sickle cell disease (Ngbolua *et al.* 2016). The authors tested the organic acid extract from the leaves of the plant, which observed the presence of alkaloids, saponins, and tannins after the chemical screening. It was shown that the drepanoid cells could regain their normal pattern. For an anti-sickling agent, it is also important to be anti-hemolytic, as the main finding of sickle cell disease is chronic anaemia. The extract of *T. riparia* showed inhibition of hemolysis of 57%. In vitro tests are essential to assess plant use. But in vivo studies are required since they provide the biotransformation effects of the components and can better evaluate their toxic effects (Okem, Finnie and Van Staden, 2012).

Free radicals are responsible for the oxidative processes of lipids, proteins and even DNA structures (Melo *et al.* 2006; Fernandez *et al.* 2017) The antioxidant activity of the plant was treated from some fractions and from TrEO. This pharmacological potential is important to interact and neutralize these radicals and thus inhibit aging and degenerative diseases (Fernandez *et al.* 2017). The TrROY isolate, being a diterpene, showed better antioxidant activity than EO and even quercetin, a flavonoid with well-known antioxidant activity (Gazim *et al.* 2014). Isolation of compounds is important, considering the fact that in the mixture of compounds that are found in essential oils for example, there may be substances that are cytotoxic, such as 9β,13β-epoxy-7-abietene (Gazim *et al.* 2014). In the isolation of some compounds. Fernandez *et al.* (2017), isolated the FR-IV which is a mixture of flavonoids (Astragalin and Luteolin). Considering the fact that the search for natural sources for antioxidants, this result is quite promising. The consumption of antioxidants is part of the treatment of Alzheimer's disease, a disorder caused by oxidative stress in human tissues and cells. However, maintaining the level of acetylcholine is the main strategy to delay the evolution of the disease. Thus, the assay with the plant extract showed potency in inhibiting acetylcholinesterase, the enzyme responsible for acetylcholine hydrolysis (Amoo *et al.* 2012).

Traditional African medicine uses the leaves of the plant to treat various infectious diseases. *T. riparia* and derivatives have shown potential pharmacological

action on a variety of pathogens such as bacteria, fungi, protozoa as the cause of leishmaniasis and some helminths.

The factors resulting from resistance to antimicrobials are the most diverse and make the search for alternatives to new drugs necessary. The composition, popularly called false myrrh, rich in isoprenes corroborates several studies on the antimicrobial action of these compounds (Koroch *et al.* 2007; Baldin *et al.* 2018). The bacterial cell wall has a lipophilic characteristic, EOs have the same characteristic, the affinity between them causes the compound to cross the cytoplasmic membrane causing cell wall disruption (Bhardwaj *et al.* 2013; Baldin *et al.* 2018). The antimicrobial activity of TrEO has been reported in recent years as activity against the main bacteria causing tooth decay, *Streptococcus mutans*, which when applied topically decreases the microorganism's adherence to teeth (Melo *et al.* 2015a). Season differences studied by Gazim *et al.* (2010) showed that *S. aureus* and *B. subtilis* were the most sensitive microorganisms, within the 9 pathogenic bacteria studied, and that the best susceptibility results occurred with leaves collected in the summer. The abandonment of the existing treatment for Tuberculosis makes new strains of *M. tuberculosis* resistant (Medeiros and Andrade 2008). Susceptibility to TrEO led Baldin *et al.* (2018) tested the potential of EO and also of the TrROY isolate against the microorganism and both obtained satisfactory results.

The effects of the plant on fungi known as dermatophytes were treated. They cause infections of the skin, hair and nails, and they grow optimally in subtropical regions, in hot and humid places (Kakande *et al.* 2019). Treatment for this type of fungus usually involves a very long time, so there is a need to find new alternatives, considering that many of the treatments used have created resistance. Most studies brought disk diffusion assays, as it is a simple test and allows the testing of various substances, using this method the plant extract showed inhibition on the hyphae growth phase (Endo *et al.* 2015), that is, the antifungal interrupts the initial stages by limiting the sporulation capacity, making it more vulnerable (Kakande *et al.* 2019). Both EO, the plant extract, and the isolate 8(14),15-sandaracopimaradiene-7alpha,18diol. showed excellent activity against *Candida albicans*, which is of great value considering that this fungus, due to its genetic flexibility, ends up becoming less susceptible to treatments already available (Buwan and Van Staden, 2006; Okem *et al.* 2012). In one of the studies, the main causative agents of respiratory infections were reported, such as *Cryptoccocus neoformans*, the use of EOs from aromatic plants proved to be effective. Probably due to its volatility, and then its entry into cryptococcosis-affected lungs and

thus aid in fighting the disease (Viollon and Chaumont 1994; York, Van Vuuren and Wet, 2012).

In this review, some studies reported the in vitro anti-*Leishmania* potential of the T. riparia plant and its derivatives (Demarchi *et al.* 2015a, 2015b, 2016, Cardoso *et al.* 2018; Terron-Monich *et al.* 2018). Research into new drugs for the treatment of leishmaniasis has been widely encouraged (Drugs for Neglected Diseases initiative, DNDi, 2021). The current therapy for leishmaniasis is based on pentavalent antimonials and amphotericin. Despite their efficacy, these drugs present complex challenges for health, such as therapeutic failure, high toxicity and serious adverse effects, patient treatment abandonment and even death. And this is sufficient justification for researching new drug and combination options (Berbert *et al.* 2018). 2018).

Given the studies, TrEO has a great anti-leishmanial activity in vitro, possibly acting on membrane lipids that lead to apoptosis and death of the parasite. It has also been shown to be excellent in the intracellular form of infection since, at a concentration that is not cytotoxic to the cell, it has significantly reduced the infection rate (Demarchi *et al.* 2015a). In comparison, TrOY, being a diterpene isolated from EO, required a higher concentration to induce the death of *L. (L.) amazonensis*. Which showed cytotoxicity to murine macrophages, but not for human erythrocyte cells. Briefly, both TrEO and its isolate showed effects on the parasite's mitochondrial and respiratory metabolism (Demarchi *et al.* 2015a; Cardoso *et al.* 2018). In addition, the host's immune response is another mechanism that is closely linked to the course of the disease, which may or may not favour the cure of the infection (Amoo *et al.* 2012; Oliveira *et al*. 2014 and Demarchi *et al.* 2015a).

Thus, the studies conducted by Demarchi *et al.* showed the in vitro immunomodulatory effects of TrEO and Terron-Monich *et al*. (2019) of TrROY. The effect has been attributed to complex mixtures of biologically active compounds that may confer protection against infectious and some non-infectious diseases (Adams 1995; Demarchi *et al.* 2015b). One of the most studied compounds with leishmanicidal and immunomodulatory potential is TrROY, a diterpene.

Also, anthelmintic activity was reported (Melo *et al.* 2015b; Okem, Finnie and Van Staden, 2012; Van Puyvelde *et al.* 2018). It is clear in the literature that drugs against infectious agents are becoming less and less effective, given the resistance of microorganisms. This fact led to the study of the effects of TrEO on *S. mansoni* by Melo *et al.* (2015b), the causative agent of Schistosomiasis, a tropical disease that affects millions of people. The study revealed that TREO was effective against the viability of

adult worms and new eggs grown. Two of the studies used *Caenorhabditis elegans* as a population, as it is an ideal nematode for studies due to its easy cultivation, which makes the assay cheaper and faster. Since it is also susceptible to most of the anthelmintics used. The plants most commonly used for the treatment of stomach pains in South Africa were tested. The extract from the leaves of *T. riparia* made with ethyl acetate was the highest activity against *C. elegans*. A promising result since helminth infections cause stomach pain (Okem *et al.* 2012). Identification and isolation of the anthelmintic compound were performed by Van Puyvelde *et al.* (2018), diterpenediol 8 (14), 15-sandaracopimaradiene-7alpha, 18-diol. This indicated activity very similar to the previous study, requiring that no extract contain large amounts of this isolate (Van Puyvelde *et al.* 2018). The mechanisms of this activity have not been elucidated, but as characteristic features of the compounds allowed interaction with the cell membrane affecting metabolic pathways (Knobloch *et al.* 1989; Melo *et al.* 2015b). These studies support the use of traditional medicine as an anthelmintic (Van Puyvelde *et al.*, 2018).

Other activities found for a plant were the high acaricide potential on the tick *Rhipicephalus (Boophilus) microplus*, an important agent that causes damage to livestock in South America, causing damage in the affected areas. It is an excellent candidate for the control of strains resistant to pesticides already used (Gazim *et al.* 2011). The repellent activity of EO was tested against the *An. gambiae* mosquito, the main vector of malaria on the African continent. Due to the fact that the repellents found are mostly synthetic in character, which can cause some toxicity in animals and humans. The effects of EO were close to synthetic repellents, which is a promising result considering that the compound has less effect on the user (Omolo *et al.* 2004).

T. riparia plant use has been reported as traditional Asian and mainly African medicine, since its effectiveness in the treatment of infectious diseases. The use of the infusion of leaves, as it is commonly prepared, indicates that there is a possibility of use since there was no denaturation of the compounds, not losing their activity (Ndiku and Ngule 2014).

Strengths and limitations

This review followed the most recommended protocols for systematic and scoping reviews, yet the selection of studies was carried out blindly and independently by two reviewers. All discrepancies were resolved by experts. Among the limitations of this review, we highlight that the most databases were investigated, and an analysis of the risk of bias was not performed yet.

5. CONCLUSIONS

The *T. riparia* plant has been usually obtained from subtropical regions, such as Brazil and Africa. The extracts, essential oil and isolates from leaves were mainly studied to investigate in vitro actions on pathogens and cells, and in vivo effects. *T. riparia* and derivatives have shown potential pharmacological action on a diversity of pathogens (protozoa, funghi, bacteria and helminths), insects and larval, also antioxidant and immunomodulatory effects. The therapeutic use of this plant and its derivatives to treat infectious and inflammatory diseases was potential, but limited since regarding the cytotoxicity and few studies in vivo and humans published.

Contributors

CLC, TP and IGD performed the literature research, designed the data extraction form, and performed data extraction and data analysis. JV, AAB and IGD critically reviewed the analyzed data. All wrote the paper. JV and IGD critically reviewed subsequent drafts. All authors approved the final version of the manuscript for submission. All authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Attachments

Figure 1. Flow diagram of study selection for this review.

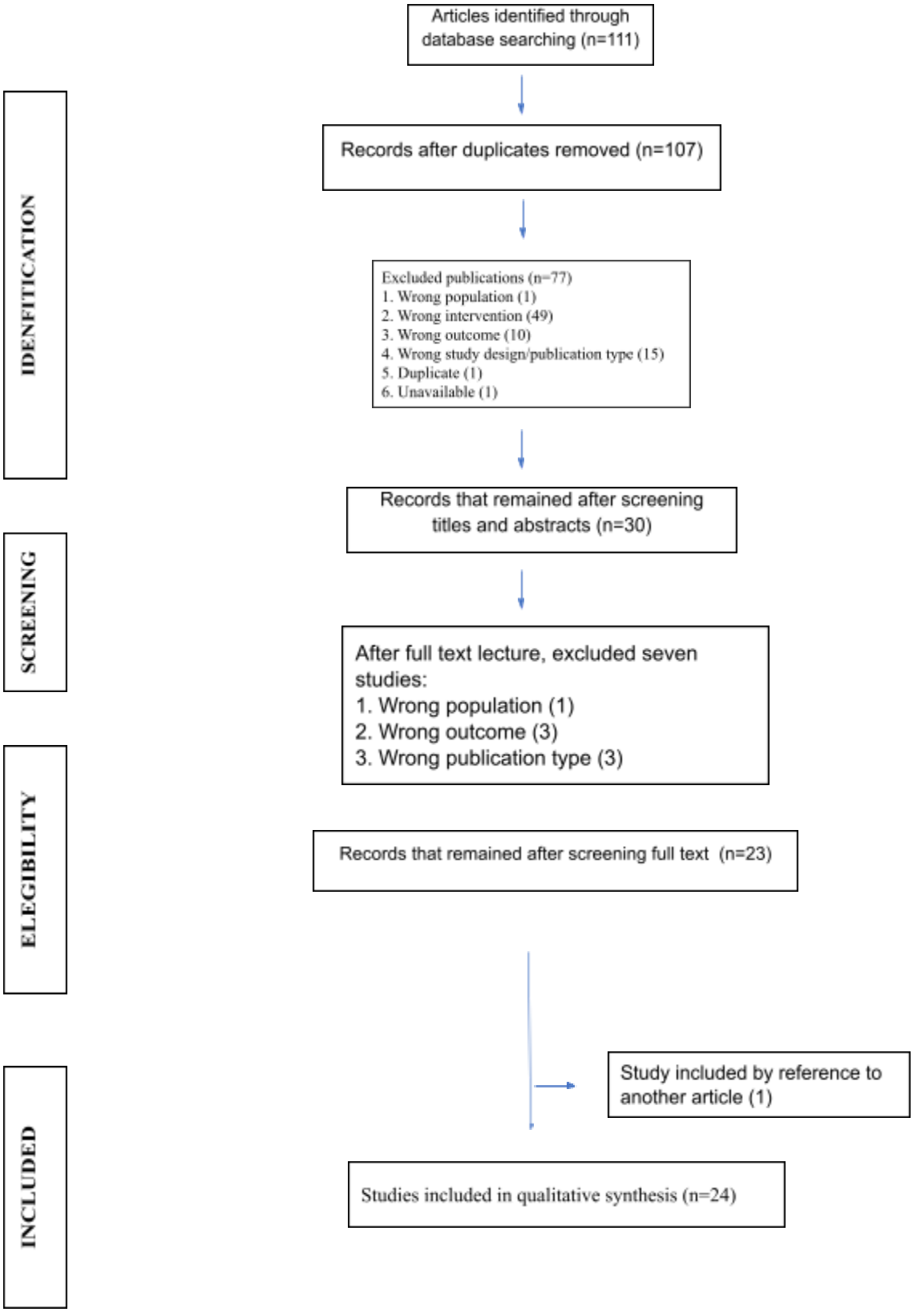

Table 1. Summary of descriptive characteristics of the included articles (n=24).

Study / Country	Part used and Derivatives	Study design	Aim of study	Population (cepas/strain, cells, animals, human)
Amoo *et al.* 2012/South Africa	Leaves (extracts)	In vitro	To investigate 21 commonly used South African medicinal plants for their phytochemical, antioxidant and AChE-inhibitory properties after 12 or 16 years storage in comparison to freshly harvested material.	Not applied (NA)
Baldin et al. 2018/ Brazil	TrEO and TrROY (isolated the *T. riparia* leaves)	In vitro	To evaluate the *In vitro* cytotoxicity and activity of TrEO and 6,7-dehydroroyleanone pure compound against *M. tuberculosis* H37Rv and susceptible and resistant clinical isolates.	1. *Bacillus cereus*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*. 2. *M. tuberculosis* H37Rv and 19 *M. tuberculosis* clinical isolates (seven susceptible to anti-TB drugs, six isoniazid monoresistance and six MDR); 3. Murine peritoneal macrophages (BALB/c mice)
Cardoso et al. 2018/Brazil	Leaves (TrEO obtained in spring, summer, autumn and winter)	In vitro In vivo	To evaluate the activity of the TrEO obtained in different seasons against *L. (L.) amazonensis* In vitro, and in BALB/c mice to determine its potential for leishmaniasis treatment.	In vitro; 1. *L. (L.) amazonensis* promastigote (strain); 2. Human erythrocyte (type O+); 3. J774.A1 macrophages; 4. Peritoneal macrophages were obtained from BALB/c mice; 5. intracellular amastigotes. In vivo: 6. Female BALB/c mice infected
Demarchi et al. 2015a/Brazil	TrEO from fresh leaves and TrROY isolated from EO	In vitro	To evaluate the potential of TrEO and TrROY isolated from *T. riparia* on *L. (L.) amazonensis* promastigote and amastigote forms, cytotoxicity on human erythrocytes and murine macrophages, nitric production and inducible nitric oxide synthase (iNOS) mRNA expression.	1. Human Erythrocyte (type O+); 2. Murine peritoneal macrophages (BALB/c mice) 3. Promastigote forms of *L. (L.) amazonensis* 4. Amastigote forms of *L. (L.) amazonensis*
Demarchi et al. 2015b/Brazil	TrEO from the fresh leaves	In vitro	They studied the immunomodulatory effects of TrEO on murine macrophages.	1. Peritoneal macrophages
Demarchi et al. 2016/ Brazil	TrEO from the fresh leaves	In vitro	To evaluate the effects of TrEO on cytokine production by peritoneal fluid cells that were infected with *L. (L.) amazonensis*, demonstrating antileishmanial activity and immunomodulatory effects of TrEO.	1.Murine Peritoneal fluid cells (BALB/c mice) 2. Resident peritoneal murine cells (BALB/c mice) 3. *L. (L.) amazonensis*
Endo et al. 2015/Brazil	Leaves hydroalcoholic extracts from *T.riparia*	**In vitro**	To evaluate the anti-dermatophytic activity of *R. officinalis*, *P. guajava* and *T. riparia* hydroalcoholic extracts.	*Trichophyton rubrum*, *T. mentagrophytes* and *Microsporum gypseum*
Fernandez et al. 2017/Brazil	Leaves extract (CE) and fractions (FR)	In vitro	To evaluate the antioxidant activity by methods of DPPH, b-carotenelinoleic acid, FRAP and total phenols, and the antimicrobial activity by broth microdilution method of the crude extract (CE) and fractions (FR) of the *T. riparia* by classical chromatography	*Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus* , *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhimurium*, and *Aeromonas hydrophila*
Gazim et al. 2010/Brazil	TrEO from the fresh leaves.	In vitro In vivo	To investigate and now report the chemical composition of the essential oil isolated from fresh leaves of *T. riparia* cultivated in southern Brazil, as affected by different growing seasons, together with their anti-inflammatory and antimicrobial activities.	1.Albino Swiss mice. 2. *S.aureus, B.subtilis, E. coli,Pseudomonas aeruginosa,E. faecalis, Proteus mirabilis, K. pneumoniae* e *Salmonella* enterica) and *Candida albicans*.
Gazim et al. 2011/Brazil	TrEO of fresh leaves during summer	In vitro	To evaluate the acaricidal activity of the essential oil from fresh leaves of *T. riparia* against the acari of *R. (B.) microplus.*	300 engorged females of *R. (B.) microplus.*
Gazim et al. 2014/Brazil	TrEO from leaves, fractions 16 and 17	In vitro	To describe the isolation and identification of a new compound from *T. riparia* essential oil, and corresponding In vitro antioxidant and cytotoxic activities	Tumor cells MDA-MB-435 (human breast carcinoma), HCT-8 (human colon), SF-295 (human nervous system) and HL-60 (human promyelocytic leukemia).
Kakande et al. 2019/ Uganda	Ethanolic Crude Leaf Extract of *T. riparia*	In vitro	To aim at determining the prevalence of dermatophytosis and antifungal activity of ethanolic crude leaf extract of *T. riparia* against dermatophytes isolated from patients attending Kampala International University Teaching Hospital, Uganda	*T. tonsurans*, *T. mentagrophyte*, and *M. audouinii*
Melo et al. 2015a/Brazil	Fresh leaves	In vitro	The chemical composition and antimicrobial activity of the essential oil of *T. riparia* leaves (TrEO) grown in Southeastern Brazil against a representative panel of cariogenic bacteria.	*Streptococcus* (*S.salivarius*, *S. sobrinus*, *S. mutans*, *S. mitis*, *S. sanguinis*) and *Lactobacillus casei*
Melo et al. 2015b/Brazil	TREO was obtained from fresh leaves	In vitro	In vitro anthelmintic effects of TrEO on *S. mansoni* adult worms and eggs, as well as its cytotoxicity against V79 mammalian cells.	*Schistosoma mansoni* adult worms and eggs
Ndiku and Ngule 2014/ Kenya	extract of leaves	In vitro	To analyze the phytochemical and antibacterial activity of hot – aqua extract of *Tetradenia riparia* leaves.	*Serratia liquefaciens*, *Salmonella typhi, Proteus vulgaris*, *E.coli*, *Enterobacter aerogenes* and *Bacillus cereus*

Ngbolua et al. 2016/Democratic Rep.of the Congo	extract of leaves	In vitro	To investigate the in vitro antisickling and direct antibacterial activities of the anthocyanins and organic acids extracted from *G. punctata* and *T. riparia*.	1. red blood cells; 2.*Staphylococcus aureus* (S. aureus ATCC 25923, MRSA ATCC 1625, MSSA ATCC 5668) and *E. coli* (E. coli ATCC 25922) strains
Ngule et al. 2014/Kenya	fresh leaves	In vitro	To analyze the antibacterial activity phytoconstituents of *T. riparia* plant.	*B. cereus, Serratia liquefactions, Serratia marcescens, Salmonella typhi, E. coli, Proteus vulgaris*, *Staphylococcus epidermidis*
Okem, Finnie and Van Staden, 2012/ South Africa	The plant extracts with EtOAc, EtOH and water. The extract of *T.riparia* was made from the leaves.	In vitro	To evaluate the efficacy of seven South African medicinal plants used in traditional medicine for the treatment of stomach-related ailments. The plant extracts were screened for antibacterial and antifungal activities against some of the pathogenic agents known to cause stomach-related ailments in immunosuppressed patients. Plant extracts were also screened for anthelmintic and cyclooxygenase-inhibitory (anti-inflammatory) activities. The genotoxicity test was done to establish the safe use of the plant extracts as therapeutic agents.	1.*E coli*, *Staphylococcus aureus* and *Enterococcus faecalis*; 2.*Candida albicans*; 3. *Caenorhabditis elegans;* *4.*solution of COX-1 and -2 enzymes; *5.Salmonella typhimurium* tester strains TA98, TA100 and TA1537 without metabolic activation
Omolo et al. 2004/Kenya	TrEO was obtained from leaves	In vivo	To report the repellent properties of the essential oils of 6 African plants, those of their constituents and synthetic blends of selected constituents against An. gambiae s.s., one of the principal Afro-tropical malaria vectors.	Six human volunteers were selected from those who showed mild or no allergic reaction to mosquito bites or candidate oils.
Sabela et al. 2017/	Nanoparticles (AgNPs) from aqueous extracts of *I. riparia* leaf and Ilex Mitis root bark with diterpenes (DTPs) and saponins (SPNs)	In vitro	To evaluate the antioxidant activity on ABTS and inhibitory effect on human embryonic kidney cells in a concentration dependent manner.	Assay on human embryonic kidney cells
Terron-Monich et al. 2018/ Brazil	TrROY	In vitro	To investigate the effects of TrROY on cytokine production by murine macrophages that were infected with *L. (L.) amazonensis*	Peritoneal macrophages were obtained from BALB/c mice
Van Puyvelde et al. 1986/Rwanda	8(14),15-Sandaracopimaradiene-7alfa,18-diol was isolated from the chloroform extract from the leaves of *T. riparia*	In vitro	To report the antimicrobial activity of the new diterpenediol, i.e. (14),15-sandaracopimaradiene-7alfa,18-diol.	*Bacillus subtilis*; *Enterobacter cloaceae* ; *E. coli*; *Klebsiella pneumoniae*; *Mycobacterium smegmatis*; *Proteus vulgaris* ; *Pseudomonas aeruginosa* ; *Pseudomonas solanacearum. Salmonella typhimurium* ; *Serratia marcescens*; *Shigella dysenteriae*; *foccus aureus* and *Streptococcus pyogenes*. *Allescheria boydii;Aspergillus flaws; Candida albicans; Epidermophyton flocosum; Microsporum ferrugineum: Microsporum canis; Microsporum gypseum; Paecelomyces; Trichophyton mentagrophytes; Trichophyton schoenleinii and Microsporum breuseghenii*.
Van Puyvelde et al. 2018/	Leaves	In vitro	To identify the compounds active against helminths from *T. riparia*.	N2 wild-type *Caenorrhabitis elegans*
York et al. 2012/South Africa	Extract from the leaves and TrEO	In vitro	To determine the antimicrobial efficacies of 30 plant species (independently and combinations) used for respiratory related infections in rural Maputaland.	*Cryptococcus neoformans*, *Klebsiella pneumoniae*, *Moraxella catarrhalis*, *Mycobacterium smegmatis* and *Staphylococcus aureus*

Y=Yes; N=Not; NR= not reported; TrEO= *Tetradenia riparia* essential oil; AmB= Amphotericin B; EO= essential oil; IL= interleukin; NO=nitric oxide; ; MIC= Minimum inhibitory concentration (MIC); CAMBH= Calcium (Ca2+) and magnesium (Mg2+) adjusted (CAMHB) medium; qPCR= quantitative polymerase chain reaction; CC50= cytotoxicity concentration 50; Ferric Reducing/Antioxidant Power= FRAP acaricidal test= AIT XTT= ; GAPDH=Glyceraldehyde- 3 phosphate dehydrogenase; Sickle cell Disease= SCD; Ethyl acetate =EtOAc; ethanol =EtOH minimum lethal concentration = MLC; 6,7-dehydroroyleanone (TrROY).

Table 2. Summary of pharmacological potential of the included articles (n=24).

Study / Country	Pharmacological Assays	Methods	Conclusion
Amoo et al. 2012/South Africa	1. Antioxidant assay; 2. Acetylcholinesterase inhibitory activity	Colorimetric assays (1a. DPPH free radical scavenging activity; 1b. β-Carotene-linoleic acid model system (oxidative destruction of carotene in the emulsion); 2. Acetylcholinesterase inhibitory activity (colorimetric method)	The medicinal plants can retain their biological activity after prolonged storage under dark conditions at room temperature. The high antioxidant activities of stable bioactive compounds in these medicinal plants offer interesting prospects for the identification of novel principles for application in food and pharmaceutical formulations.
Baldin et al. 2018/ Brazil	1. Antimicrobial assay 2. Anti-*M. tuberculosis* activity 3. Cytotoxicity assay on murine peritoneal macrophages	1.Broth microdilution method using CAMHB medium 2. Resazurin Microtiter Assay Plate (REMA). 3. Colorimetric assay (Alamar Blue).	TrEO isolated from leaves of *T. riparia* and the pure compound TrROY display good activity against *M. tuberculosis* clinical isolates, including MDR isolates, with low cytotoxicity to murine macrophages. The TrROY compound is a potential candidate for anti-TB drugs. Furthermore, TrROY showed no dramatic effects on bacteria pertaining to human microbiota, an extremely important fact when studying a new compound with activity against pathogens.
Cardoso et al. 2018/Brazil	In vitro (1. Antileishmanial activity (promastigote); 2. Haemolytic activity (HA) assay; 3. J774.A1 macrophages Cytotoxicity;4. Murine macrophages Cytotoxicity 5. Nitrite determination; 6. Activity against intracellular amastigotes In vivo (7. Activity anti-*Leishmania* in BALB/c mice infected)	In vitro (1. Microscopic counting in a Neubauer chamber; 2. Colorimetric assay (absorbance of the supernatant was determined at 550 nm for estimation of haemolysis); 3. XTT colorimetric assay; 4. Trypan Blue assay (microscopic counting); 5. Griess colorimetric assay; 6. Microscopic counting In vivo: 7. Parasite load in lymph nodes and spleen (microscopic counting using microdilution in plate)	TrEO shows potential for development of a new and safer drug with fewer side effects for the treatment of leishmaniasis.
Demarchi et al. 2015a/Brazil	1. Hemolysis assay (Erythrocyte toxicity); 2. Macrophages cytotoxicity; 3. Antileishmanial assays on promastigote forms: a) Viability; b) Growth inhibition c) Ultrastructural alterations 4. Antileishmanial assays on amastigote forms; 5. Nitrite assay 6. iNOS mRNA expression	1. Colorimetric assay; 2. XTT colorimetric assay; 3a. XTT; 3b. Microscopic counting; 3c. Transmission electron microscopy; 4. Microscopic counting; 5. Colorimetric assay (Griess); 6. Semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR)and flow cytometry	TrROY and mainly TrEO promoted the *Leishmania* death, and TrROY showed loss of toxicity to erythrocytes cells. Other compounds derived from T. riparia and the essential oil could be explored to develop a new alternative treatment for leishmaniasis.
Demarchi et al. 2015b/Brazil	1.Immunomodulatory assay 2.Cytokine mRNA expression 3.Macrophage cytotoxicity	1 e 2.Reverse-transcriptase polymerase chain reaction (cytokines mRNA expression) and flow cytometry; 3.Cytotoxicity in macrophages was determined using XTT method	The essential oil from *T. riparia* may be an alternative therapy for carcinogenic, autoimmune, and infectious diseases in which cellular responses are critical for their resolution.
Demarchi et al. 2016/ Brazil	1.Citotoxity; 2. Immunomodulatory effects; 3. Antileshmanial activity;	1. It was determined using a colorimetric cell viability XTT method; 2. Reverse-transcriptase polymerase chain reaction (cytokines mRNA expression) and flow cytometry; 3. to placed in 24-well culture plates/ PCR	The antileishmanial and immunomodulatory effects of TrEO support the use of traditional medicines, such as T. riparia, for the treatment of parasitic infections. TrEO may be an alternative leishmaniasis therapy when considering its antileishmanial and immunomodulatory effects.
Endo et al. 2015/Brazil	Antifungal assays	1.Disc diffusion method; 2.Fluorescence microscopy 3.Scanning electron microscopy	The study reports that leaves from *R. officinalis* and *T. riparia* contain antifungal bioactive compounds. Hydroalcoholic extracts from both species proved to be effective against dermatophytes, inhibiting fungal growth and causing morphological alterations in their hyphae. This supports their use in folk medicine to treat skin disorders. These species are potential sources for the development of antifungal treatment strategies.

Fernandez *et al.* 2017/Brazil	1.Antioxidant assays 2.Antimicrobial assays	1.total phenols; DPPH; Beta-Carotene/Linoleic Acid System Method; Method of Ferric Reducing/Antioxidant Power (FRAP); 2. To determined by the broth microdilution method using Mueller Hinton Broth (Difco Laboratories Sparks) with CAMHB, per recommendation of the Clinical and Laboratory Standards Institute	To demonstrate that extract fractionation was extremely important to detect the presence of fractions with antioxidant and antimicrobial activity superior to CE of myrrh. The CE and FR-IV presented promising results of antioxidant potential and FR-I in the antimicrobial action on the Gram-positive bacteria strains.
Gazim *et al.* 2010/Brazil	1.The antimicrobial activity 2.The anti-inflammatory/ analgesic activity	1.Disc diffusion method 2the mouse constriction test	The analgesic activity of the oil was not affected by this seasonal variation. These differences can be attributed to the seasonal changes in rainfall. The oil from T. riparia showed good analgesic and antimicrobial activities. affected by this seasonal variation.
Gazim et al. 2011/Brazil	1. acaricidal test 2. larval packet test (LPT)	Counting stereomicroscope	The study shows that the essential oil of *T. riparia* grown in southern Brazil showed high acaricidal activity against the tick R. (B.) microplus. The results showed that the essential oil of T. riparia is a promising biocontrol candidate for use against pesticide-resistant strains of *R. (B.) microplus.*
Gazim et al. 2014/Brazil	1. Antioxidant Activity 2.Cytotoxicity Activity	1. DPPH radical scavenging, β-carotene-linoleic acid and ABTS assays. 2. MTT colorimetric technique	By fractionating the oil, it was possible to identify two unpublished compounds: 1 with high cytotoxic potential and 2 with high antioxidant potential. These results provided additional perspectives to the evaluation of new compounds with pharmacological activity. In future research we intend to expand the studies on biological activities of these compounds and other isolated fractions from the essential oil of T. riparia to develop new applications in pharmacology.
Kakande et al. 2019/ Uganda	Antidermatophytic Activity	1. Modified agar well diffusion method 2.Tube dilution method	The findings of this study reported the presence of dermatophytes causing dermatophytosis among patients attending KIU-TH. The results of the current study showed that *T. riparia* leaves ethanolic crude extract has antidermatophytic activity against tested dermatophytes.
Melo et al. 2015a/Brazil	antimicrobial assays	broth microdilution method in 96-well microplates (CLSI 2009) using DMSO.	The essential oil of T. riparia (TR-EO) displays promising antimicrobial activity against some cariogenic bacteria, including Streptococcus mutans, which is one of the main causative agents of dental caries. The TR-EO chemical composition is slightly different from that reported in previous studies. Taken together, our results suggest that this essential oil might be promising for the development of new oral care products. Further studies aiming to identify the active chemical constituents of TR-EO are underway
Melo et al. 2015b/Brazil	1. Schistosomicidal assays 2. Cytotoxic activity	1. MTT assay; 2. in vitro Toxicology Colorimetric Assay Kit (XTT; Roche Diagnostics)	TrEO was effective against *S. mansoni* adult worms and inhibited egg development. Taken together, the present results suggest that the schistosomicidal activity of this essential oil deserves further investigation to identify the chemical constituents that elicit this activity.
Ndiku and Ngule 2014/ Kenya	Antibacterial activity	Mueller Hinton agar plates	The plants *Tetradenia riparia* has shown great potency as a source of new antibiotics in the treatment against all the infections caused by the microorganisms tested. The results in this research are a clear indication that the plant extracted in the traditional way can be used for the treatment of various diseases affecting human beings today. It is worthy to mention that the action of the plant against the microorganisms might be due to a synergistic effect of two or more compounds found in the plant. From the results obtained in this research we can recommend the plant for the treatment of all the ailments caused by the bacteria used.
Ngbolua et al. 2016/Democratic Republic of the Congo	1.Antisickling and hemolytic experiments; 2.Antibacterial activity	(1.1)Hypoxic induced hemolysis assay; (1.2)Osmotic fragility test; (1.3)ITANO test; (1.4) Met-hemoglobin reducing test. 2. microdilution method in 96-well microplates diluted with Mueller Hinton Broth	To evaluated the chemical composition of Garcinia punctata and *Tetradenia riparia* and the antisickling and antibacterial effects of anthocyanins and organic acids extracted from the above mentioned plant species and suggests that extracts from these plants could be a source of naturally occurring antisickling and antibacterial agents. This scientific based evidence supports the possibility of using *G. punctata* and *T. riparia* extracts as an affordable medicine for managing SCD and allied diseases in DRC. It is therefore necessary to screen these extracts for their in vitro and in vivo toxicological effects in order to guarantee their harmlessness
Ngule et al. 2014/Kenya	Antibacterial activity	1.The bioassay was done using well diffusion method	1.The presence of important pharmacological phytoconstituents and the antibacterial activity observed in the plant fresh leaves water extract indicates the medicinal importance of *Tetradenia*

			riparia. The current study may be a partial scientific justification of the plants use in the treatment against the infections caused by all the tested microorganisms. The isolation of active ingredients, identification of their structural make up, mode of action and their effect in the in vivo environment remains inevitable in order to provide the safety precautions (if any) which are needed when using this plant. Further research on the isolation of the active ingredients needs to be done in the future.
Okem et al. 2011/ South Africa	1.Antibacterial activity; 2. Antifungal activity; 3. Anthelmintic activity; 4. Anti-inflammatory assay; 5. genotoxicity potential;	1 and 2. microdilution assay 3. *in vitro* determination of free-living nematode larvae viability; 4. Radioactivity assay 5. *Salmonella* microsome assay	The results of pharmacological screening presented in this study to some extent validate the efficacy of the selected medicinal plants used in treating stomach-related ailments. However, other aspects of stomach-related ailments such as gastric ulcers, motility disorders, indigestion and the infections of the lower gastro-intestinal tract were not covered in this study. The genotoxicity test showed that none of the plant extracts were mutagenic towards the Salmonella tester strains. However, further studies such as toxicity and the use of metabolizing enzymes are needed to ascertain the safety of the plant metabolites on long-term consumption. Phytochemical analysis revealed different classes of secondary metabolites in the plant species studied. This indicates the type of bioactive compounds that could be responsible for the antimicrobial, anthelmintic and anti-inflammatory activities.
Omolo et al. 2004/Kenya	Mosquito repellency assays of essential oil, individual constituents and selected blends	Insects counting	*C. newii* and *P. marrubioides* for evaluation as sources of fumigants (thermal expulsion and potted plants) in reducing man-vector contact in screenhouse experiments and in rural homesteads. The more potent individual terpenoids are being assessed in different formulations for personal protection.
Sabela et al. 2017/	1.Cytotoxic activity 2.Antioxidant activity	1.MTT assay 2.Ultraviolet–visible spectroscopy with ABTS	Based on the cytotoxicity and antioxidant activity, it is fair to report that these plant extracts have potential reducing and capping agents as they retain chemical properties on the surface of the nanoparticles.that these plant extracts have potential reducing and capping agents as they retain chemical properties on the surface of the nanoparticle.
Terron-Monich et al. 2018/ Brazil	1.immunomodulatory effects; 2. anti-Leishmanial activity	1.Cytokine Magnetic 10-Plex Panel Multiplex Mouse Kit, this method is based on a suspension that contains beads, in which encoded fluorescent beads have cytokine capture antibodies to bind the proteins; 2. Microscopic counting	TrROY may be a promising anti-Leishmania agent that can stimulate the protective immune response against intracellular pathogens and inhibit undesirable immune reactions. TrROY may be an alternative therapeutic agent for leishmaniasis. Also, considering the modulation of IL-4 released by TrROY, this diterpene should be evaluated in other parasitic diseases that induce TH2 bias. The expression and activity of arginase should also be investigated to elucidate the immunomodulatory effects of TrROY on this pathway. Additional in vitro and in vivo tests should be performed to confirm the antiLeishmania and immunomodulatory potential of TrROY.
Van Puyvelde et al. 1986/Rwanda	antimicrobial activity	It was determined by the liquid dilution method.	*T. riparia* is one of the most popular medicinal plants in Rwanda. Previously, several new substances have been isolated from the leaves of this plant, including a new diterpene diol, i.e. 8(14),15-sandaracopimaradiene-7a, 18diol. This new diterpene diol exhibits significant antimicrobial activity against several bacteria and fungi. The minimum inhibitory concentration (MIC) of the substance for microorganisms which were inhibited ranged from 6.25 to 100 pg/ml.
Van Puyvelde et al. 2018/	1.Anthelmintic activity	1.The anthelmintic assay was carried out in a 96-well microplate	They identified the bioactive compound from Tetradenia riparia responsible for its anthelmintic activity: 8(14),15-sandaracopimaradiene-7α,18-diol.
York et al. 2012/South Africa	Antimicrobial activity	Microdilution assay	1.Although this in vitro study supports the traditional use of some plants independently and in combination for the treatment of respiratory ailments in rural Maputaland, results demonstrate that the traditional selection of plants in higher frequency do not necessarily correlate with higher antimicrobial efficacy.

Y=Yes; N=Not; NR= not reported; TrEO= *Tetradenia riparia* essential oil; AmB= Amphotericin B; EO= essential oil; IL= interleukin; NO=nitric oxide; CC50=; MIC= Minimum inhibitory concentration (MIC); CAMBH= Calcium (Ca^{2+}) and magnesium (Mg^{2+}) adjusted (CAMHB) medium; qPCR= quantitative polymerase chain reaction CC50= cytotoxicity concentration 50; Ferric Reducing/Antioxidant Power= FRAP acaricidal test= AIT XTT= ; GAPDH=Glyceraldehyde- 3 phosphate dehydrogenase; Sickle cell Disease= SCD; Ethyl acetate =EtOAc; ethanol =EtOH minimum lethal concentration = MLC; 6,7-dehydroroyleanone (TrROY).

Supplemental file Table S1 – PICOS strategy.

PICOS	Inclusion Criteria	Exclusion criteria
***P**opulation*	Human In vivo: any animals In vitro: any cells/other (protozoa, funghi, bacteria, virus)	In silica
***I**ntervention*	*Tetradenia riparia* (plant and its derivatives)	Synthetic compounds
***C**omparison*	Placebo, another drug or combination, reference drugs	No control
***O**utcomes*	Pharmacological and toxicological effects (e.g.: antioxidant, antimicrobial, immunomodulator, adverse effects)	In silico, pharmacokinetic and pharmacodynamic outcomes
***S**tudy design*	Experimental studies: in vitro, in vivo and clinical trials	Review, letter, conference abstract, editorial, guideline
Research question:	What are the major pharmacological and toxicological effects of the plant *Tetradenia riparia* and its derivatives?	

PICOS strategy for studies of intervention (Needleman IG, 2002; Higgins JPT, Green S. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. England: John Wiley & Sons, Ltd; 2011).

Supplementary Table S2. Search Strategy.

Search	Query/ Pubmed	LILACS	Google
Block 1 Plant #1	**Tetradenia riparia* (free term) OR *Iboza* OR *Moschosma*	Lamiaceae (descriptor)	allintitle:tetradenia riparia filetye:pdf
Block 2 Pharmacology #2	Pharmacology cell, cultured Biological assay	NOT review	-
Block 3	#1 AND #2	#1 AND #2	-

Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) Checklist

SECTION	ITEM	PRISMA-ScR CHECKLIST ITEM	REPORTED ON PAGE #
TITLE			
Title	1	Identify the report as a scoping review.	9
ABSTRACT			
Structured summary	2	Provide a structured summary that includes (as applicable): background, objectives, eligibility criteria, sources of evidence, charting methods, results, and conclusions that relate to the review questions and objectives.	11
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known. Explain why the review questions/objectives lend themselves to a scoping review approach.	12,13
Objectives	4	Provide an explicit statement of the questions and objectives being addressed with reference to their key elements (e.g., population or participants, concepts, and context) or other relevant key elements used to conceptualize the review questions and/or objectives.	13
METHODS			
Protocol and registration	5	Indicate whether a review protocol exists; state if and where it can be accessed (e.g., a Web address); and if available, provide registration information, including the registration number.	13
Eligibility criteria	6	Specify characteristics of the sources of evidence used as eligibility criteria (e.g., years considered, language, and publication status), and provide a rationale.	14,15
Information sources*	7	Describe all information sources in the search (e.g., databases with dates of coverage and contact with authors to identify additional sources), as well as the date the most recent search was executed.	15,61
Search	8	Present the full electronic search strategy for at least 1 database, including any limits used, such that it could be repeated.	15
Selection of sources of evidence†	9	State the process for selecting sources of evidence (i.e., screening and eligibility) included in the scoping review.	15,16
Data charting process‡	10	Describe the methods of charting data from the included sources of evidence (e.g., calibrated forms or forms that have been tested by the team before their use, and whether data charting was done independently or in duplicate) and any processes for obtaining and confirming data from investigators.	16
Data items	11	List and define all variables for which data were sought and any assumptions and simplifications made.	16
Critical appraisal of individual sources of evidence§	12	If done, provide a rationale for conducting a critical appraisal of included sources of evidence; describe the methods used and how this information was used in any data synthesis (if appropriate).	-
Synthesis of results	13	Describe the methods of handling and summarizing the data that were charted.	16

RESULTS			
Selection of sources of evidence	14	Give numbers of sources of evidence screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally using a flow diagram.	17,54
Characteristics of sources of evidence	15	For each source of evidence, present characteristics for which data were charted and provide the citations.	17-39
Critical appraisal within sources of evidence	16	If done, present data on critical appraisal of included sources of evidence (see item 12).	-
Results of individual sources of evidence	17	For each included source of evidence, present the relevant data that were charted that relate to the review questions and objectives.	17-39
Synthesis of results	18	Summarize and/or present the charting results as they relate to the review questions and objectives.	2, 17-39
DISCUSSION			
Summary of evidence	19	Summarize the main results (including an overview of concepts, themes, and types of evidence available), link to the review questions and objectives, and consider the relevance to key groups.	39-45
Limitations	20	Discuss the limitations of the scoping review process.	46
Conclusions	21	Provide a general interpretation of the results with respect to the review questions and objectives, as well as potential implications and/or next steps.	46
FUNDING			
Funding	22	Describe sources of funding for the included sources of evidence, as well as sources of funding for the scoping review. Describe the role of the funders of the scoping review.	46

JBI = Joanna Briggs Institute; PRISMA-ScR = Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews.
* Where *sources of evidence* (see second footnote) are compiled from, such as bibliographic databases, social media platforms, and Web sites.
† A more inclusive/heterogeneous term used to account for the different types of evidence or data sources (e.g., quantitative and/or qualitative research, expert opinion, and policy documents) that may be eligible in a scoping review as opposed to only studies. This is not to be confused with *information sources* (see first footnote).
‡ The frameworks by Arksey and O'Malley (6) and Levac and colleagues (7) and the JBI guidance (4, 5) refer to the process of data extraction in a scoping review as data charting.
§ The process of systematically examining research evidence to assess its validity, results, and relevance before using it to inform a decision. This term is used for items 12 and 19 instead of "risk of bias" (which is more applicable to systematic reviews of interventions) to include and acknowledge the various sources of evidence that may be used in a scoping review (e.g., quantitative and/or qualitative research, expert opinion, and policy document).

From: Tricco AC, Lillie E, Zarin W, O'Brien KK, Colquhoun H, Levac D, et al. PRISMA Extension for Scoping Reviews (PRISMAScR): Checklist and Explanation. Ann Intern Med. 2018;169:467–473. doi: 10.7326/M18-0850.