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Insights on Pharmacological Properties of *Combretum leprosum* Mart.

José Carlos da Silveira Pereira[1], Carlos Campos Câmara[2], Luana Cassandra Breitenbach Barroso Coelho[3] and Michele Dalvina Correia da Silva[1*]

[1]Laboratório de Biologia Celular e Molecular, Centro de Ciências Biológicas e da Saúde, Universidade Federal Rural do Semi-Árido (UFERSA), Mossoró, Rio Grande do Norte, Brasil.
[2]Laboratório de Fisiologia e Farmacologia Experimental, Centro de Ciências Agrárias, Universidade Federal Rural do Semi-Árido (UFERSA), Mossoró, Rio Grande do Norte, Brasil.
[3]Laboratório de Bioquímica de Proteínas, Departamento de Bioquímica, Centro de Biociências, Universidade Federal de Pernambuco, Recife, Pernambuco, Brasil.

Authors' contributions

This work was carried out in collaboration between all authors. Authors JCSP and MDCS managed the literature searches. Authors MDCS and LCBBC designed and managed the study performed. Authors JCSP and CCC conducted the experimental research and performed the statistical analysis. Author JCSP wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Combretum leprosum Mart. is a plant widely used in folk medicine; several studies revealed anti-inflammatory, antinociceptive, toxic, antiproliferative, antibacterial, antiparasitic, neuroprotective and gastroprotective effects, among other properties. However, there are no reports on evaluation of preparations obtained from this species' tissues, exploring biological responses of macromolecular components, such as proteins, common active agents. Proteins, such as lectins, promote important pharmacological results due to specific interaction with carbohydrates or

**Corresponding author: E-mail: micheledalvina@ufersa.edu.br;*

glycoconjugates. In this work, a protein fraction obtained from a saline extract of *C. leprosum* leaves was evaluated for anti-inflammatory potential in a paw edema assay induced by carrageenan in Wistar rats. A significant anti-edematous effect was observed after 3 h (100 mg protein/kg) and 6 h (30 mg/kg and 100 mg/kg). The saline extract and protein fractions showed lectin activity, inhibited by D-(+)-mannose, D-(+)-trehalose dihydrate, D-(+)-galactose and D-fructose. Conclusively, leaves of *C. leprosum* contained proteins and lectin(s), promising anti-inflammatory agents. A vision of *C. leprosum* pharmacological properties is also approached.

Keywords: Combretum leprosum; mofumbo; edema; inflammation; lectin.

1. INTRODUCTION

The family Combretaceae consists of 20 genera, totaling approximately 600 species. The major genera are *Combretum* and *Terminalia*, which comprise 370 and 200 species, respectively [1,2]. Specimens of this family are distributed in the tropics and subtropics, especially in Africa [3].

Phytochemical studies involving *Combretum* species are continuously performed; new compounds are discovered and characterized, such as cycloartanes, flavonoids, saponins, coumarins, triterpenes, combretastatins, ellagic acid derivatives, anthracene glycosides, aromatic dibenzyls, stilbenes, phenanthrenes, lignans and amino acids [3,4,5,6]. Some biological properties have already been associated with these compounds [3,5,7,8,9,10,11].

Some species of the genus *Combretum* have been evaluated for potential pharmacological effects. Among them, the species *C. leprosum* Mart. stands out due to its diverse, proven medicinal properties. Different compounds obtained from *C. leprosum* were pharmacologically evaluated. Arjunolic acid (representing 65% of the components from root ethanolic extracts) and (-)-epicatechin (present in bark hydroalcoholic fraction) have anti-inflammatory and antinociceptive effects [5,7]. A triterpene (lupane) termed 3β,6β,16β-trihydroxylup-20(29)-ene (TTHL) is already widely characterized as an anti-inflammatory agent and has antileishmania effect [9]. Bark hydroalcoholic extract (containing triterpenes, flavonoids, tannins and saponins) revealed gastroprotective effects [6]. In addition to *C. leprosum* compounds, some constituents produced by microorganisms associated with this species have pharmacological effects, such as endophytic fungi with antifungal and anticancer properties [8]. These and other pharmacological studies approached in this review demonstrate the medical potential of *C. leprosum* due to secondary metabolites.

However, the literature does not report the macromolecular composition of *C. leprosum* tissues, such as proteins, potential biologically active agents. In this review, we describe general pharmacological properties of *C. leprosum*. A protein fraction obtained from leaves of this species revealed anti-inflammatory action and lectin activity.

2. *Combretum leprosum*: MOLECULAR CONSTITUTION AND PHARMACO-LOGICAL PROPERTIES

C. leprosum is a species native from the Caatinga Brazilian biome, commonly found in the northern and northeastern states of Brazil, as well as in the states of Mato Grosso, Mato Grosso do Sul, in semi-deciduous forests. This plant is popularly known as *mufumbo*, *mofumbo* or *cipoaba* [5,12] and several of its tissues, such as leaves and flowers, are widely used in varied forms in folk medicine to cure pathologies. Preparations are used to prevent skin eruptions, to cleanse wounds [13], as an expectorant and antitussive [14], sedative, antidiarrheal, to treat bronchitis, influenza, whooping cough, sweating, diphtheria, heartburn, as a soothing or hemostatic agent [15,16] and even as an antidote to snake venoms [13,17].

C. leprosum is a scandent shrub or liana, 2-3 m tall, with lepidote indument of scaly, shiny, hyaline or whitish trichomes, which cover the stem, branches, leaf buds, flowers and fruits. It has opposite, petiolate leaves, which are ovate or oblong and with an acute apex. Its flowers are subsessile, fusiform, with 4 petals and 8 stamens. It has betuloid and winged fruit, with an oval seed including 4 grooves, accompanying the shape of the dark brown fruit [18]. Fig. 1 shows macromorphological characteristics of *C. leprosum*.

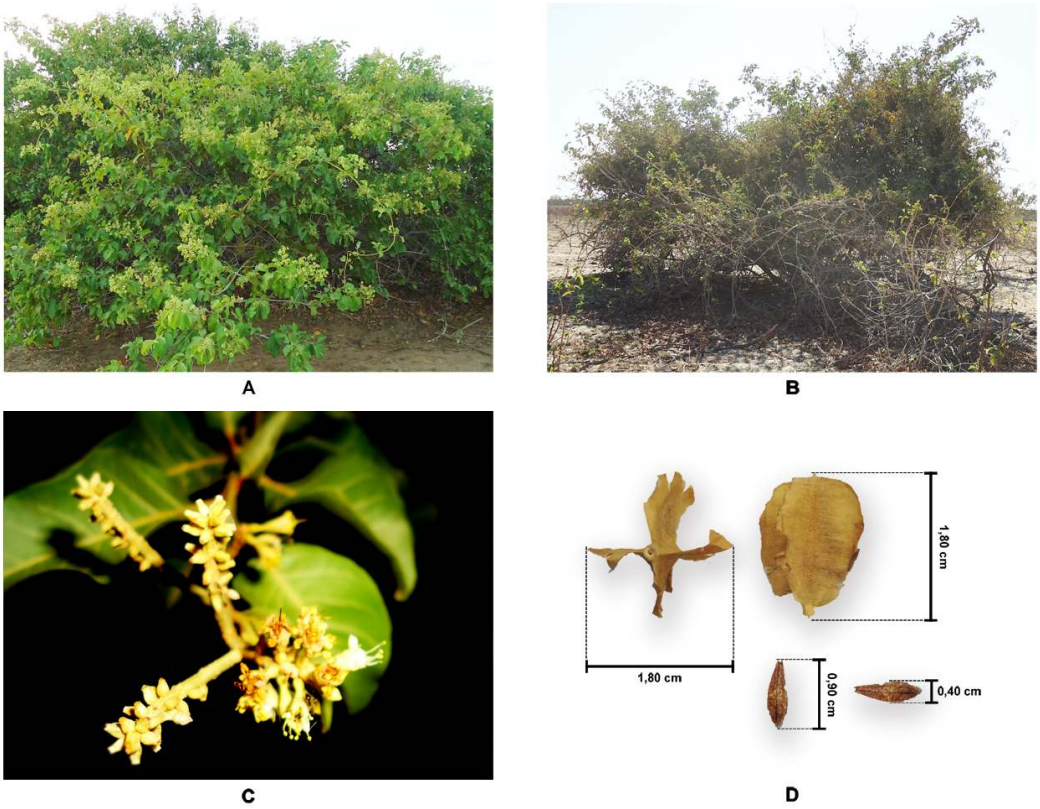

Fig. 1. Macromorphological characteristics of *Combretum leprosum* (Russas City, State of Ceará, Brazil, 4°50'28.6"S 37°54'05.5"W): (A) Shrub during the rainy season; (B) Shrub during the dry season; (C) Inflorescence; (D) Fruit and seed

There is a range of research exploring pharmacological properties of alcoholic, hydroalcoholic extracts and isolated compounds of this species, focusing on the action of its secondary metabolites. Studies using ethanolic extracts of leaves and roots of *C. leprosum* allowed the isolation, identification and characterization of the chemical structure by X-ray crystallography from the triterpene (lupane) TTHL, in addition to other compounds such as arjunolic acid, mollic acid and glycosylated flavonoids (3-O-methylquercetin and quercetin) [4]. The ethanolic extract of *C. leprosum* leaves consists of monosaccharides (80%), triterpenes (10%), minor oligosaccharides (5%) and fatty acids (3%) [5]. The main components found in the ethanolic extract of *C. leprosum* bark are triterpenes, flavonoids, tannins and saponins [6]. Some of these substances have important anti-inflammatory, antinociceptive and antiproliferative biological activities. Fig. 2 shows molecules found in *C. leprosum* with pharmacological applications.

3. ANTI-INFLAMMATORY ACTIVITY

Inflammation is a body response to various stimuli and may be associated with pathologies [19,20,21]. Several developed drugs interfere to minimize organ damage; these medications are anti-inflammatories, which may be steroidal or non-steroidal. Steroidal anti-inflammatory drugs (glucocorticoids) act, for the most part, by inhibiting various inflammatory proteins [22] while non-steroidal anti-inflammatory drugs (NSAIDs) may be nonselective or selective inhibitors of cyclooxygenase [23]. Some natural compounds act effectively as anti-inflammatories.

Administration of an ethanolic extract of *C. leprosum* flowers in a model of acute inflammation induced by a phorbol ester (12-O-tetradecanoylphorbol-13-acetate, TPA) in mice promoted a dose-dependent inhibition of edema, as well as a reduction of myeloperoxidase activity (a direct marker of neutrophil infiltration), of tissue IL-6 levels and cell infiltration, followed

by histological analysis [13]. Accordingly, administration of the extract in a chronic inflammatory model induced by crotonic oil also promoted the reduction of edema and cell infiltration, as well as epidermal hyperproliferation and expression of proliferating cell nuclear antigen (PCNA), indicating an anti-inflammatory effect [13].

TTHL from *C. leprosum* flowers was able to reduce levels of the proinflammatory cytokines IL-1β and TNF-α in a murine model of carrageenan-induced peritonitis [24], although it did not stimulate the production of TNF-α in human peripheral blood mononuclear cell cultures [25] and IL-10 levels [25]. These data, associated with a reduction in migration of total leukocytes (mainly neutrophils), reinforce the antinociceptive and anti-inflammatory effects, both in a murine model of visceral nociception induced by acetic acid and in a carrageenan-induced peritonitis model [24], indicating that these effects may be related to inhibition of the glutamatergic system [24,26].

In a model of experimental wounds in mice, TTHL lupane contributed to the formation of new blood vessels structuring the extracellular matrix, accelerating epithelization, with an evident anti-inflammatory response [27]. In addition, an aqueous suspension of lyophilized hydroalcoholic extract from *C. leprosum* bark promoted relaxation of isolated arterial rings of different animal species, requiring an influx of Ca^{2+}, potentially responsible for the release of relaxation factors in endothelial cells [28].

The ethanolic extract of *C. leprosum* roots and isolated arjunolic acid, orally administered, reduced the paw edema induced by carrageenan, showing anti-inflammatory activity [29]. Arjunolic acid also reduced ear edema induced by topical application of arachidonic acid (AA), with no effect on ear edema promoted by topical application of phorbol ester, suggesting that the compound affects the pathway of AA metabolism by the action of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes [5].

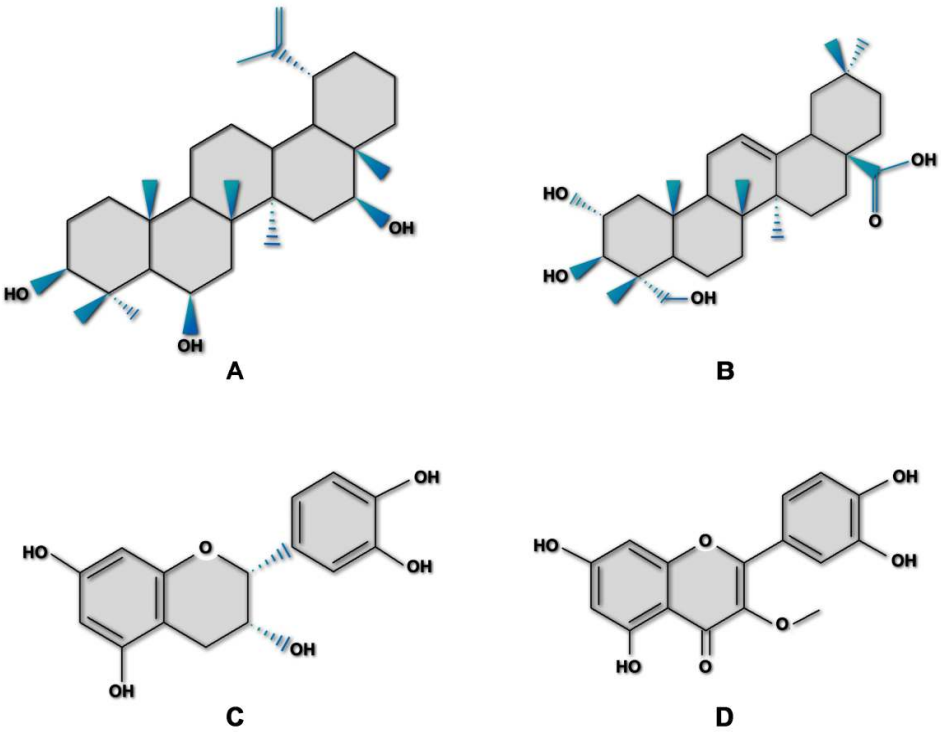

Fig. 2. Molecular structure of compounds isolated from *Combretum leprosum*. (A) 3β,6β,16β-trihydroxylup-20(29)-ene, TTHL; (B) 2α,3β,23-trihydroxyolean-12-en-28-oic acid, arjunolic acid; (C) (-) epicatechin, EPI; (D) 3-O-methylquecertin

The protein fraction of *C. leprosum* leaves with lectin activity was obtained by saline fractionation with ammonium sulfate at 30-60% saturation of the crude extract (protein quantified according to Lowry et al. [30]). When tested by intraperitoneal administration in the paw edema model induced by carrageenan (according to Landucci et al. [31]) it was able to significantly reduce edema, especially during the late phase (in which edema is rich in leukocyte infiltrate), promoting a dose-dependent response. After the phlogistic stimulus, there was a significant difference compared to control group, in the treatments using 100 mg/kg after 3 h and 30 mg/kg as well as 100 mg/kg after 6 h, which edema reduced 66.5%, 64.8% and 84.8%, respectively. Carrageenan-induced paw edema is a model of acute biphasic inflammation [32]. The initial phase (1-2 h) of this assay is characterized by the release of histamine, 5-hydroxytryptamine (serotonin, 5-HT) and bradykinin, followed by a late phase (3-4 h) sustained by the release of prostaglandins [32].

Studies have shown the anti-inflammatory potential of plant lectins through *in vivo* models of inflammation induced by the phlogistic agent carrageenan [33,34,35,36,37,38]. Carrageenan stimulates resident cells to release chemotactic inflammatory mediators, increasing migration of leukocytes, especially neutrophils [32]. Results obtained in the present study suggest that F2 may have acted on inflammatory mediators, as well as may have inhibited the final stage of leukocyte migration from the bloodstream to the site of inflammation, which involves rolling and cell adhesion, both mediated by lectin-carbohydrate interaction [39]. Plant lectins have been described as potential inhibitors of leukocyte rolling and adhesion, and as anti-inflammatory molecules [38,40].

In inflammation processes, cyclooxygenase (prostaglandin endoperoxide synthase) catalyzes the first step in the formation of prostaglandins [41], important for maintenance of the late phase of inflammation [32]. A lectin isolated from *C. mkuzense* bark was able to inhibit 82% of cyclooxygenase activity [41]. Thus, this biosynthetic pathway may have been affected by F2, inhibiting the late phase of acute inflammation model. Inhibitors of prostaglandin biosynthesis are potential anti-inflammatory agents [41].

Another example of species anti-inflammatory potential was the action of neutralizing toxic activities (formation of edema, myotoxicity, hemorrhage, proteolytic and hyaluronidase activity) of *Bothrops jararacussu* and *B. jararaca* venoms. These effects were promoted by the ethanolic extract of *C. leprosum* roots and by isolated arjunolic acid, evaluated through *in vitro* and *in vivo* assays at different concentrations and protocols (pre-incubation, pretreatment and post-treatment) [42]. These results corroborate with folk medicine, since *C. leprosum* is used to combat the effects of snakebites [13].

4. ANTINOCICEPTIVE ACTIVITY

The sensory system of nociception includes primary neurons capable of detecting a variety of stimuli, forming the "pain pathway" [43]. Pain can traditionally be classified as somatic, visceral or neuropathic and involves several mechanisms, which need the use of a combination from different medications [44,45,46]. In addition to the inflammatory drugs mentioned above, opioid analgesics aid in the reduction of nociception [46,47]. The analgesic effect of opioids acts on the pain modulation system through endogenous opioid peptides and their receptors [45]; *C. leprosum* has compounds capable of reducing nociception.

The hydroalcoholic extract of *C. leprosum* bark showed antinociceptive effect in animals submitted to hotplate and formalin assays in neurogenic and inflammatory phases [12]. Similarly, oral administration of ethanolic extract from *C. leprosum* flowers increased latency time in the hotplate test, and reduced nociception induced by acetic acid, capsaicin, glutamate or formalin in neurogenic and inflammatory phases [1]. Non-selective or selective receptor antagonists, naloxone, pindolol, WAY100635 and ketanserin reversed the extract effect on glutamate-induced nociception. Flower molecular constituents may promote interactions with opioidergic and serotonergic systems blocking nociception [1].

Similarly, the hydroalcoholic fraction of bark ethanolic extract from *C. leprosum* also showed antinociceptive effect in chemical models of glutamate-, capsaicin- and formalin-induced nociception [48]. This fraction effect, which involves the participation of serotonergic, adrenergic and nitrergic receptors, may be partially attributed to its isolated flavonoid, (-)-epicatechin [7,48] (PPE, Fig. 2C).

EPI, in a chemical model with glutamate, had its antinociceptive effect reversed by different antagonists: naloxone (2 mg/kg), glibenclamide

(antagonist of ATP-dependent K^+ channels, 2 mg/kg), ketanserin (0.3 mg/kg), pindolol (1 mg/kg), yohimbine (alpha-2-adrenergic receptor antagonist, 1 mg/kg) and atropine (muscarinic antagonist, 0.01 mg/kg) [7]. The reversal effect of EPI by these antagonists suggests the involvement of opioid receptors and potassium channels sensitive to ATP and action on the serotonergic, adrenergic and cholinergic systems in the antinociceptive effect of EPI [7].

Similarly, TTHL isolated from *C. leprosum* flowers inhibited somatic nociception induced by intragastric administration of acetic acid, when applied 1 h after induction (ID_{50} value of 0.15 mg/kg) [24]. There was also inhibition of somatic nociception induced by intragastric administration of formalin - in the neurogenic (ID_{50} value of 108 mg/kg) and inflammatory phases (ID_{50} approximately 30 mg/kg) - and of glutamate, 20 µmol/paw (ID_{50} value of 19 mg/kg) in animals pretreated with TTHL 1 h prior induction [49]. Pretreatment with triterpene showed a greater antinociceptive effect (ID_{50} value of 5.6 mg/kg) when evaluated in animals submitted to half the dose of phlogistic agent (glutamate, 10 µmol/paw) [1].

TTHL antinociceptive effect involved opioidergic and serotonergic systems, evidenced by its ability to reverse the effect caused by antagonists' naloxone, CTOP (selective µ-opioid receptor antagonist), nor-binaltorphimine (selective antagonist of κ-opioid receptor), naltrindole (selective δ-opioid receptor antagonist), p-chlorophenylalanine methyl ester (inhibitor of serotonin synthesis), WAY100635 and ketanserin [49]. TTHL antinociceptive action is also related to activation of $G_{i/o}$ protein and ATP-sensitive potassium channels. Its effect was inhibited when animals were pretreated intrathecally with the pertussis toxin (PTX, $G_{i/o}$ protein inactivator) and with charybdotoxin antagonists (K^+ channel blockers of Ca^{2+}-dependent large channels), tetraethylammonium (voltage-dependent K^+ channel blocker) and glibenclamide (ATP-dependent K^+ channel blocker) [49].

In contrast, no association was observed between the antinociceptive effect of flower ethanolic extract with the nitrergic system (in the presence of L-arginine) or with 5-HT_3 receptors (with ondansetron) [1]. In addition, no association was found between the antinociceptive effect of EPI plus the purinergic (assessed in the presence of caffeine) and nitrergic systems (with L-arginine) or including 5-HT_3 receptors (evaluated with ondansetron) [7]. Similarly, the antinociceptive effect promoted by TTHL was not reversed by L-arginine, ondansetron (5-HT_3 receptor antagonist) or apamine (Ca^{2+}-dependent K^+ channel blocker) [49]. Therefore, flower ethanolic extract, hydroalcoholic fraction of ethanolic bark extract, TTHL and EPI (obtained from flowers and bark of *C. leprosum*, respectively) neither presented depressant effects on the central nervous system nor a muscle relaxant effect [1,7,48,49].

TTHL caused dose-dependent inhibition on a murine model of glutamate-induced nociception, when administered intraperitoneally, intraplantarly and intrathecally. TTHL antinociceptive effect may be related to modulation of glutamatergic system [50].

Another compound with antinociceptive potential is arjunolic acid, isolated from root ethanolic extract of *C. leprosum*, which inhibited the activity of butyryl- and acetylcholinesterase enzymes [5].

5. TOXICITY AND ANTIPROLIFERATIVE EFFECT

Toxic effects may alter the mitotic index (MI), total number of cells dividing in cell cycle, resulting in increased MI [51,52,53], leading to disordered cell proliferation and formation of tumor tissues [53]; or decreased MI [53,54], characterizing chemical action on growth and development of exposed organism. Antiproliferative factors are necessary, compounds that interfere in cellular development, which act as efficient chemotherapeutic agents [55].

The ethanolic extract of *C. leprosum* flowers and their isolated flavonoids, 3-O-methylquercetin (5,3'-dihydroxy-3,7,4'-trimethoxyflavone) and quercetin 3,7-dimethyl ether (5,3',4'-trihydroxy-3,7-dimethoxyflavone), showed protective effect against hydrogen peroxide (H_2O_2) in strains of *Saccharomyces cerevisiae* [10]. 3-O-Methylquercetine revealed a better antioxidant action, which may be associated with the activation of the hydroxyl group at the 3' position in the presence of the methoxy group at the 4' position from the B-ring of the molecule [10].

These flavonoids did not induce mutagenesis in strains of *S. cerevisiae*, although a mutagenic effect was promoted by the ethanolic extract (500 µg/mL) [10]. TTHL obtained from this extract induced mutagenicity in the non-mutant strain

XV185-14c (WT) of *S. cerevisiae*. Mutant strains of *S. cerevisiae* EG118 (*sod1Δ*), EG110 (*sod2Δ*) and EG133 (*sod1Δsod2Δ*) deficient in superoxide dismutase (an antioxidant defense) were hypersensitive to TTHL [56].

The ethanolic extract and its isolated flavonoids were not cytotoxic to lung fibroblasts of Chinese hamsters (cell line V79), but quercetin 3,7-dimethyl ether (75 µg/mL) significantly increased DNA damage index [10], suggesting that 3-O-methylquercetin is most suitable for pharmacological use, based on its antioxidant effect, low cytotoxicity and lack of genotoxicity [10].

TTHL induced a time-dependent and concentration-dependent growth inhibition of human cell lines MRC5 (normal lung fibroblasts), MCF-7 (breast adenocarcinoma), HepG2 (hepatoma), T24 (bladder cancer), CACO-2 (colorectal adenocarcinoma), HCT116 (colorectal carcinoma) and HT29 (colorectal adenocarcinoma) [56]. The greatest antiproliferative activity of TTHL was promoted in MCF-7, with 120 h of incubation (IC_{50} value of 0.30 µg/mL). The IC_{50} (1.36 µg/mL) and IC_{80} (3.70 µg/mL) observed with 24 h incubation of MCF-7 cells with TTHL induced apoptosis in 14% and 52% of the cells, respectively, as detected by increased cleavage/activation of caspase-9 and production of intracellular reactive oxygen species (ROS). The potential of this compound in altering cellular redox balance may play a role in its toxicity [56].

In addition to evaluation of flower preparations for cytotoxic effects, *C. leprosum* bark was also used to estimate mice and rats toxicity. Bark hydroalcoholic extract showed acute toxicity when orally and intraperitoneally administered in male mice [12]. This extract showed no reproductive toxicity in female rats; it did not promote estrogenic or antiestrogenic activity or toxicity in the embryonic stages (implantation, fertilization or organogenesis), with no fetal mortality [57].

6. ANTIBACTERIAL AND LEISHMANICIDAL EFFECT

Antibacterial are compounds that can inhibit growth (bacteriostatic) or kill bacteria (bactericidal) [58,59]; the latter may be applied in the treatment of short-term [60] and long-term infections [61] and in immunocompromised patients [62]. However, bacterial resistance has increased due to frequent and improper use, requiring new alternatives for bacterial control [58,59].

The ethanolic extract of *C. leprosum* leaves and isolated TTHL inhibited growth and formation of biofilms from the Gram-positive bacteria *Streptococcus mutans* UA159 (ATCC 700610) and *Streptococcus mitis* (ATCC 903), but they did not affect the Gram-negative bacteria *Pseudomonas aeruginosa* (ATCC 10145) or *Klebsiella oxytoca* (ATCC 13182) [63]. TTHL had the best values of minimum inhibitory concentration (MIC = 7.8 µg/mL) and minimal bactericidal concentration (MBC = 15.6 µg/mL) for *S. mutans* and *S. mitis* [63]. The lupane MIC also inhibited, respectively, 97% and 90% the biomass formation of these bacteria [63]. In an acute toxicity analysis using *Artemia nauplii*, LC_{50} of TTHL was 98.19 µg/mL, greater than its efficient concentration against the bacteria [63].

In this context, leishmanicidal drugs lead to considerable clinical advances, reducing parasitic load; however, they are toxic and do not promote cure [64,65]. Pentavalent antimonials are primarily indicated for the treatment of leishmaniasis; drugs such as amphotericin B and pentamidine are also effective [64,65,66,67]. Natural products have fostered the development of new leishmanicidal drugs [9,64,68,69].

Teles et al. [68] highlighted the leishmanicidal activity of *C. leprosum* fruits and isolated lupane against *L. amazonensis* promastigotes. The leishmanicidal potential of modified triterpenes obtained from lupane isolated from *C. leprosum* is lost with the substitution of hydroxyl groups by acetyl, and maintained with the substitution of hydroxyl groups by carbonyl groups [68].

Lupane (5 µg/mL, 109 µM) showed no toxicity to murine peritoneal macrophages [9]. In contrast, it showed greater toxicity to peripheral blood mononuclear cells (PBMC) at concentrations higher than 1.5 µg/mL [25]. TTHL viability from intracellular amastigote form of *L. amazonensis* was evaluated. It promoted a reduction in the rate of infected macrophages and in number of amastigote forms, showing a time-dependent effect which inhibited infection (83.8%, 96 h of incubation) a superior inhibitory activity when compared to Glucantime® (48 h of incubation), a drug used to treat leishmaniasis [9].

Lupane, even at cytotoxic concentrations (6 µg/mL), did not interfere in topoisomerase activity isolated from PBMC [25]. However, through a bioinformatics analysis, a perfect

coupling and a strong binding affinity between TTHL and *Leishmania braziliensis* DNA topoisomerase were demonstrated, suggesting the inhibition pathway in *L. amazonensis* [9]. Additionally, TTHL has its medical use reinforced by molecular docking, which observed low or no probability of inhibition from human topoisomerases I and II by lupane, corroborating with biological assays [25].

A liposome-lupane, consisting of dipalmitoylphosphatidylcholine, dipalmitoylphosphatidylserine, cholesterol (ratio of 5:1:4) and TTHL (2 mg) was prepared and tested [69]. The liposome-lupane reduced survival of *L. amazonensis* amastigotes in infected peritoneal macrophages and diminished paw lesions in infected mice [69]. Its administration increased IL-12 and reduced IL-10 levels in the supernatant of *L. amazonensis*-infected macrophages, favoring activation profile of T_H1 response [69]. The drug leishmanicidal effect stimulated immune response in macrophages; TTHL is potentially applicable as an immunostimulatory agent in other models of infection.

7. EFFECT ON THE NERVOUS SYSTEM AND GASTRIC TISSUE

TTHL promoted antinociceptive and anti-inflammatory effects related to modulation of the glutamatergic system [1,24,50]. However, its anticonvulsant effect on the nervous system is not directly associated with antioxidant or GABAergic activities, but with the neuroprotective effect of TTHL *in vitro* and *in vivo* due to its ability to maintain Na^+/K^+-ATPase activity in the face of treatment with a seizure agent [70].

The ethanolic extract of *C. leprosum* flowers, rich in TTHL, reduced motor deficits in a murine model of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [26]. This biological response may be associated with the protective effect of components from the dopaminergic system; a 2-fold increase was measured in the mRNA levels of tyrosine hydroxylase (TH) gene and dopamine transporter gene (DAT) [26].

The ethanolic extract from *C. leprosum* bark, orally administered, inhibited the appearance of gastric lesions induced by ethanol and reduced gastric lesions promoted by indomethacin [6]. The extract administered by an intraduodenal route in pylorus-linked rats revealed an increase in pH, reduction in volume and acidity of gastric juice produced, as well as an increase in mucus content in gastric wall [6]. Then the extract showed gastroprotective and antiulcerogenic effects, related to the inhibition of gastric acid secretion and increase of mucosal defensive factors such as mucus and prostaglandins [6].

8. BIOLOGICAL ACTIVITIES OF ENDOPHYTIC EXTRACTS ISOLATED FROM *C. leprosum*

Endophytes are bacteria or fungi that may have a partial or total life cycle in inter- or intracellular area of plant tissues, without any damage. Important compounds for medicine, agriculture and industry can be obtained from these microorganisms [8,71,72,73].

Endophytic fungi have been isolated from leaves of *C. leprosum*; strains identified by homology of the 18S rRNA in database were *Fusarium oxysporum* JN604548 (99%), *Hypocrea koningii* AJ301990 (99%), *Aspergillus oryzae* DQ155287 (98%) and *Fusarium solani* EGY1 JQ837837 (99%) [8]. Extracts from isolated fungi were evaluated against the human pathogenic fungi *Candida albicans*, *Candida krusei*, *Candida glabrata*, *Candida guillermondi*, *Candida tropicalis*, *Cryptococcus neoformans* and *Trichophyton rubrum* [8]. *F. oxysporum* extract promoted antifungal activity against *C. glabrata* (MIC = 4 µg/mL), *C. neoformans* (MIC = 62.5 µg/mL) and *T. rubrum* (MIC = 62.5 µg/mL) [8].

Extracts of these fungi were also evaluated for their cytotoxic and antiproliferative potential on HeLa carcinogenic strains (cervical cancer cells), ECV304 (bladder carcinoma), B16F10 (mouse skin melanoma), J774 (histiocytic sarcoma), P388 (lymphoid leukemia), Jurkat (leukemic T lymphoblasts) and K562 (chronic myeloid leukemia) [8]. The best cytotoxic effect was promoted by the extract of *A. oryzae*, acting on J774 (IC_{50} value of 0.8 µg/mL), Jurkat (IC_{50} value of 0.89 µg/mL), and solid ECV304 tumors (IC_{50} value of 3.08 µg/mL) as well as HeLa (IC_{50} value of 2.97 µg/mL) [8]. In addition, this extract induced rapid morphological change in murine thyme endothelioma cells (tEnd.1), causing cell rounding, suggesting an inhibition of tumor vessel development [8].

9. ARE THERE LECTINS IN *C. leprosum* CONSTITUTION?

There is a gap in the knowledge of *C. leprosum* macromolecular composition, such as proteins,

that may be responsible for many effects in folk medicine. However, we demonstrated that protein preparations of *C. leprosum* showed hemagglutinating activity (Table 1), inhibited by carbohydrates (Table 2), indicating the presence of lectins among its constituents.

Carbohydrates act as intermediates in cellular communications in many biological systems, thus influencing phenomena such as differentiation, proliferation, and cellular interactions, in physiological and pathological conditions. Lectins stand out defined as proteins or glycoproteins of non-immune origin that bind to carbohydrates and are capable of agglutinating cells and precipitating polysaccharides and glycoconjugates, through reversible carbohydrate-binding sites [75]. Thus, the lectin property of binding carbohydrates and recognize the structure of oligosaccharides conjugated to proteins or lipids on cell surfaces characterize them as valuable tools for the study of cellular physiology and interactions.

The extensive research on the biological properties of lectins, especially those of plant origin, has demonstrated a diversity of pharmacological actions, among them the antinociceptive, pro- and anti-inflammatory effects [76]; depending on the route of administration used, lectins may induce pro- or anti-inflammatory responses. Lectin proinflammatory effects result from indirect mechanisms, depending on the activation of macrophages, probably associated with release of neutrophil chemotactic factors [36]. The anti-inflammatory effects of lectins are commonly evaluated by inhibition of paw edema [77]; such effects have been attributed to the competitive blocking of selectin-binding sites in leukocyte membrane and/or endothelial cells [78] or even to inhibition of leukocyte infiltration [76,79].

Lectins are present in *C. mkhuzense*, another species of the genus *Combretum* also used in traditional medicine [80]; *C. leprosum* leaves possess lectin(s) (Tables 1 and 2); different extracts and compounds isolated from distinct tissues promote anti-inflammatory action [13,24,25,27,28] and biological applications. Lectins or other tissue proteins may promote biological activities through specific mechanisms of action. The popular use of various *C. leprosum* preparations to treat pathologies [14] reinforce the need to purify and evaluate proteins from the plant.

Table 1. Hemagglutinating activity, protein quantification and specific hemagglutinating activity of preparations obtained from leaves of *Combretum leprosum*

Samples	Human erythrocyte (type)	HA (title^{-1})	Protein (mg/mL)	SHA (HA/mg/mL)
E	A	2048	14.98	137
	B and O	1024		68
F1	AB	4096	45.51	90
F2	AB	1024	22.11	46
F3	AB	2048	15.86	129

Leaf meal was extracted in 0.15 M NaCl (10% w/v) filtered and centrifuged; extract (E) was submitted to protein fractionation with ammonium sulfate at saturations of 30%, 30-60% and 60-90%; each saturation was centrifuged, resuspended and dialyzed against 0.15 M NaCl to obtain protein fractions (F1, F2 and F3, respectively). Preparations were submitted to protein quantification [30] and hemagglutinating activity (HA) assays using glutaraldehyde treated human erythrocytes (ABO system); specific HA (SHA) corresponds to HA titer divided by protein concentration in mg/mL [74]

Table 2. Inhibition of hemagglutinating activity from leaf preparations of *Combretum leprosum*

Samples	Carbohydrate inhibitors
F1	D-(+)-mannose (25 mM), D-(+)-trehalose dihydrate (25 mM)
F2	D-(+)-mannose (25 mM)
F3	D-(+)-mannose (25 mM), D-(+)-trehalose dihydrate (25 mM), D-(+)-galactose (50 mM), D-fructose (50 mM)

Leaf meal was extracted (E) in 0.15 M NaCl (10% w/v) followed by filtration and centrifugation; E was submitted to protein fractionation with ammonium sulfate at saturations of 30%, 30-60% and 60-90%, respectively. Each saturation was centrifuged, resuspended and dialyzed against 0.15 M NaCl to obtain F1, F2 and F3, respectively. Preparations were submitted to hemagglutinating activity (HA) inhibition assays to confirm lectin presence [74]. D-(+)-Glucose, D-(+)-galactose, D-fructose, D-(+)-mannose, D-(+)-trehalose dihydrate and α-lactose monohydrate (concentrations between 25 and 200 mM, in 0.15 M NaCl) were used with glutaraldehyde treated human erythrocytes, AB type (2.5% v/v, 0.15 M NaCl). Titers obtained were compared with HA titers in the absence of carbohydrate to determine inhibitory carbohydrates and lowest inhibitory concentrations

10. CONCLUSION

C. leprosum is an important species of the *Caatinga* biome and a rich source of metabolites with pharmacological applications, mainly terpenes and derivatives. The biotechnological studies of the plant is desirable, especially protein constituents little reported in the literature.

ETHICAL APPROVAL

All authors hereby declare that the "Principles of Laboratory Animal Care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws, where applicable. All experiments have been examined and approved by the appropriate ethics committee (Comissão de Ética em Experimentação Animal – CEEA of the Universidade do Estado do Rio Grande do Norte - UERN, approval report CEEA/UERN nº 010/2016).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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