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MICROBIOLOGY

Metabolic profile, antimicrobial and toxicity evaluation of *Azadirachta indica* roots

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ABSTRACT: *The constituents of the hydroethanolic extract of* Azadiracta indica *root were investigated using ultra-performance liquid chromatography-quadrupole-time-of-flight mass spectrometry (UPLC-QTOOF-MS^E). Acute toxicity was evaluated in an experimental animal model. We investigated the antibacterial activities of* A. indica *roots against* Salmonella typhimurium *and* Staphylococcus aureus *and the antifungal activities against strains of* Trichophyton rubrum, Candida albicans *and* Candida tropicalis. *We identified nine secondary metabolites in the hydroethanolic extract by UPLC-QTOOF-MS^E. The extract was highly effective in inhibiting the growth of* T. rubrum strains, *so it can be effective in combating the dermatophyte tested, but it had no inhibition potential on any bacterial strains or* Candida *species evaluated. It was possible to infer that the extract had no acute toxicity in relation to the animal model* Danio rerio. *Therefore, since neem has a high bioactive potential and adapts well to the climate of semiarid regions, growing this species could become a source of income for farmers by its use to produce naturals fungicide and drug, as alternatives to conventional products, which can cause microbiological resistance and/or are toxic to the environment, besides being expensive.*
Key words: Azadirachta indica, *root, toxicity, antifungal activity, antibacterial activity.*

Perfil metabólico, atividade antimicrobiana e de toxicidade das raízes de *Azadirachta indica*

RESUMO: *Os constituintes do extrato hidroetanólico da raiz de* A. indica *foram investigados por cromatografia líquida de ultra-alta performance acoplada à espectrometria de massas do tipo quadrupolo-tempo de voo (UPLC-QTOOF-MS^E). A toxicidade aguda foi avaliada em modelo animal. Investigamos as atividades antibacterianas contra* Salmonella typhimurium *e* Staphylococcus aureus *e as atividades antifúngicas contra cepas de* Trichophyton rubrum, Candida albicans *e* Candida tropicalis. *Identificamos nove metabólitos secundários no extrato etanólico por UPLC-QTOOF-MS^E. O extrato foi altamente eficaz na inibição do crescimento de cepas de* T. rubrum, *podendo ser eficaz no combate ao dermatofito avaliado, mas não apresentou potencial de inibição em nenhuma cepa bacteriana ou espécies de* Candida *avaliadas. Também foi possível inferir que o extrato não apresentou toxicidade aguda em relação ao modelo animal* Danio rerio. *Portanto, como o Neem tem alto potencial bioativo e se adapta bem ao clima das regiões semiáridas, o cultivo dessa espécie pode se tornar uma fonte de renda para os agricultores a partir da utilização da planta para produção de fungicida e/ou fármaco naturais como alternativa aos produtos convencionais, que podem causar resistência microbiológica e/ou são tóxicas ao meio ambiente, além de serem caros.*
Palavras-chave: Azadirachta indica, *raiz, toxicidade, atividade antifúngica, atividade antibacteriana.*

INTRODUCTION

Neem (*A. indica*) is a plant native to the region between India and Burma, belonging to the family *Meliaceae* (KUMAR & NAVARATNAM, 2013; ZHU et al., 2017). This species has gained global importance because it is an extremely versatile medicinal plant (DALLAQUA et al., 2013). In India, *A. indica* has been used in traditional medicine for over 2000 years because of its anti-inflammatory, anti-ulcer, anti-malarial, anti-bacterial and anti-oxidant activities (BHOWMIK et al., 2011; KURIMOTO et al., 2014). In countries where it is cultivated, studies have reported that its preparations are effective against ringworm and other fungal infections, as well as dermatitis, eczema and acne. Also, leaf cataplasms or decoctions are used against boils and the oil is used to cure skin diseases such as scrofula and indolent ulcers (BHOWMIK et al., 2011).

The compounds previously isolated and identified are divided into two broad categories:

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isoprenoids and non-isoprenoids. Among the isoprenoids are the diterpenoids and triterpenoids, containing protomeliacins, limonoids, azadirone and its derivatives, gedunin and its derivatives, vilasinin and C-seco meliacins compounds such as nimbin, salanin and azadirachtin. The non-isoprenoids include proteins (amino acids) and carbohydrates (polysaccharides), sulfur compounds, polyphenols such as flavonoids and their glycosides, coumarin, tannins, aliphatic compounds and others (MORIELLO & NISBET, 2000; COSTA et al., 2010; DAVID et al., 2017; GUPTA et al., 2017; SIVACHIDAMBARAMA et al., 2017; KUMAR et al., 2018; PASCOLI et al., 2019).

The azadiractins, preferentially reported in neem seeds, are complex limonoid tetranortriterpenoids well known to be the most active constituents contained in the species, presenting antifungal and antifeedant properties against insects (MORIELLO & NISBET, 2000; DAVID et al., 2017). Azadirachtin, who named the class, is the active principle of several neem-based products and the most studied substance of the species. Despite the wide use of this active principle, due to its complexity it has not yet been synthesized, so all products available in the market are prepared by the extraction of compounds from the plant (COSTA, 2010). Besides azadirachtin, meliantriol, limonene, odoratone and other triterpenoids are biologically active among the more than 100 compounds isolated (DELEITO & BORJA, 2008).

The search for bioactive plant species with antimicrobial action has intensified in recent years due to the increased resistance of pathogenic microorganisms against synthetic products. The specialized literature has shown that the use of crude extracts or essential oils against pathogens and phytopathogens, including viruses, is promising, and they can have fungitoxic activity, through direct antimicrobial action as an eliciting action, activating defense mechanisms in plants. Some plant extracts and essential oils are as efficient against fungi and bacteria as conventional fungicides and antibiotics, with the advantage of not being toxic to humans and the environment (CUNICO et al., 2003; SHAAPAN et al., 2021). The literature contains studies that, in addition to evaluating the bioactive potential of neem, compare the results obtained with the efficiency of commercial antibiotics or antifungals, emphasizing its high efficiency for the control of *S. typhimurium* and *S. aureus*, and antifungal activity against strains of *T. rubrum*, *C. albicans* and *C. tropicalis*.

S. typhimurium is a non-specific zoonotic bacterium responsible for causing self-limiting gastroenteritis. Furthermore, some strains of have been shown to be highly invasive, crossing the intestinal wall and reaching the systemic circulation, causing more severe infections (SANTOS et al., 2019). *S. aureus* is a Gram-positive, immobile and coagulase-positive bacterium with coccoid shape belonging to the phylum *Firmicutes*. Among the 52 species and 28 subspecies of the genus, *S. aureus* is the most clinically relevant, being found in the human commensal microbiota of the nasal mucosa in 20-40% of the general population (LEE et al., 2018). *S. aureus* is well adapted to its human host and the hospital environment, causing endocarditis, bacteremia, osteomyelitis and skin and soft tissue infections. *S. aureus* has emerged as of the main causes of infections associated with the hospital environment (TURNER et al., 2019).

Candida spp are commensal fungi of the skin, mouth and gastrointestinal tract. Despite being part of the human flora, they also have the ability to become pathogenic, causing a wide spectrum of conditions, ranging from superficial infections of the hair and nails to deadly systemic infections. The most common species are *Candida albicans, Candida glabrata, Candida parapsilosis, Candida tropicalis* and *Candida krusei*. In recent decades, *C. albicans* has been responsible for 50% of all candidemia cases. Its growth and spread are controlled by the coexistence of microbial flora, intact epithelial barriers and innate immune system defenses (PFALLER et al., 1997; BERKOW & LOCKHART, 2017).

T. rubrum is an anthropophilic fungus commonly related to the dermatophytosis known as *tinea pedis* (athlete's foot). This species of dermatophyte is atypical in animals, but due to the close contact between dogs, cats and humans, there are reports of isolation of strains in these animals, even where the owners did not suffer from athlete's foot at the time of the study (KUSHIDA & WATANABE, 1975; BALDA et al., 2004; MORIELLO et al., 2017; REIS et al., 2020). Both in humans and animals, dermatophytosis causes superficial infections and lesions of keratinized tissues such as the stratum corneum, hair and nails (CECONI et al., 2018). This type of ringworm is important in canine and feline hosts, and due to its zoonotic nature, it is naturally transmissible between animals and humans, variously through direct contact, food, water and/or the environment (ANDRADE & ROSSI, 2019).

The literature mainly elucidates the metabolic profiles of the aerial organs of the species and the bioactivity of its constituents. To the best of our knowledge, this paper reports the first study of the metabolic profile bactericidal and fungicidal activities of the hydroethanolic extract of *A. indica* roots. The constituents of the hydroethanolic root extract were investigated using ultra-performance liquid chromatography-quadrupole-time-of-flight mass spectrometry (UPLC-QTOOF-MS^E). This technique is currently considered the state of the art for compound separation and identification of substances. We investigated the antibacterial activities against *S. typhimurium* and *S. aureus* and the antifungal activities against strains of *T. rubrum, C. albicans* and *C. tropicalis*, along with the acute toxicity of the hydroethanolic extract (evaluated in an experimental animal model).

MATERIALS AND METHODS

Identification and extraction of the plant material

The roots were collected from adult plants with approximate age of 5 years, at the campus of the State University of Ceará (Universidade Estadual do Ceará - UECE), Fortaleza, Brazil (03º 43 '02' 'S and 38º 32' 35'' W). The specimen was identified by Luiz Wilson Lima-Verde of the Prisco Bezerra Herbarium of Federal University of Ceará (Universidade Federal do Ceará –UFC), Fortaleza, Brazil, on March 13, 2018, where an exsicata was deposited under number 61196.

The hydroethanolic extract was obtained from 200 g of roots previously washed in running water and dried in an oven at 40 °C. The plant material was crushed and immersed in 1 L of EtOH-H_2O (75:25, v/v) for seven days at room temperature, protected from light, with occasional stirring. The extract, passed through filter paper, was evaporated to dryness in a rotary evaporator, resulting in 1.2 g of crude extract.

Analysis by UPLC-QTOOF-MS^E

The analyses were conducted with an Acquity UPLC chromatograph (Waters, USA) coupled to a Xevo quadrupole and time-of-flight mass spectrometer (QTOF, Waters). The chromatographic runs were performed with a Waters Acquity BEH UPLC column (150 mm x 2.1 mm I.D., 1.7 µm) at 40 °C. The mobile phases were water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B), eluted with 2%-95% B (0-15 min); 100% B (15.1 to 17 min) and equilibrating with 2% B (17.1 to 19.1 min) at a flow rate of 0.4 mL min^{-1} and an injection volume of 5.0 µL. Ionization was performed with electrospray ionization source (negative mode - ESI), acquired in the range of 110-1180 Da, source temperature set at 120 °C, desolvation temperature of 350 °C, desolvation gas flow rate of 500 L h^{-1}, extraction cone of 0.5 V, and capillary voltage of 3.2 kV. Leucine enkephalin was used as lock mass. The mode of acquisition was MS^E. The instrument was controlled by the Masslynx 4.1 software (Waters Corporation).

Acute toxicity evaluation with Danio rerio

For each drug and concentration evaluated, groups of six wild adult specimens of *D. rerio* (zebrafish), aged between 60 and 90 days, were used, to which 20 µL of the drug/sample was given orally at concentrations 0.2, 0.5, 1.0, 1.5 and 2.0 mg mL^{-1}, solubilized in distilled water/Tween 80 (2% v/v). As a negative control, distilled water/Tween 80 (2% v/v) was administered, and one group received only water. After 24, 48 and 72 h, the numbers of dead animals were counted. Statistical analysis was performed by the trimmed Spearman-Karber method with 95% confidence intervals, to estimate the median lethal dose (the dose that promotes the death of 50% - LD_{50}) of the zebrafish.

Antifungal activity

The antifungal activity was evaluated by the broth microdilution technique in 96-well plates, carried out in duplicate using clinical isolates of *C. albicans, C. tropicalis* (2.5 - 5 x 10^4 CFU mL^{-1} and n=4) and *Trichophyton rubrum* (5 x 10^4 CFU mL^{-1} and n=3). Concentration ranges from 2 to 2500 µg mL^{-1} were evaluated. In the control test, the standard drugs amphotericin B and ketoconazole were used at concentrations of 0.125 to 64 µg mL^{-1}.

Initially, 100 µL of RPMI medium was added to all wells and 10 mg mL^{-1} of extracts diluted in DMSO was then added to all wells of the first column to form serial dilutions. Finally, 100 µL of the inoculum was added to the wells. The plates were covered with parafilm and incubated at 37 °C. The readings were carried out by measuring the diameters of the fungal growth inhibition zones in millimeters, after incubation of two days for the *Candida* strains and four days for the *T. rubrum* strains. The minimum inhibitory concentration (MIC), defined as the lowest concentration of extract capable of inhibiting 100% of the visible growth of the fungus, was determined by visualization as recommended by the guidelines M27-A3 from the Clinical and Laboratory Standards Institute (CLSI) and FONTENELLE et al., (2007).

The minimum fungicidal concentration (MFC) for the species was determined by sub-culturing 100 µL of the turbidity-free well solution in potato agar at 28 °C (FONTENELLE et al., 2007).

Antibacterial activity

The antibacterial activity was investigated with the strains of *S. typhimurium* ATCC 14028 and *S. aureus* ATCC 27664. The tests were performed by the disc diffusion method as described in CLSI (CLSI, 2008). The extract was evaluated at concentrations of 1.4, 5.0 and 10 mg mL^{-1}. Laborclin chloramphenicol discs (30 µg mL^{-1}) were used as positive control. Three discs for each dose of extract were prepared for each bacterium. In addition, an empty disc used to prepare the extract discs was used for control. The MIC and minimal bactericidal concentration (MBC) of each extract was determined by inhibition zones caused by the extract, measured in millimeters. The bacteria were inoculated in Petri dishes containing Mueller-Hinton agar (MH) and incubated inverted in a bacteriological oven for 24 h at 35 °C. According to this technique, a growth inhibition halo of any degree around the paper discs is considered a positive result. The experiment was performed in triplicate.

RESULTS AND DISCUSSION

Identification by UPLC-QTOOF-MS^E

Table 1 presents the compounds tentatively identified in positive mode by their retention time, fragmentation pattern, molecular formula and error (in ppm) in studies reported previously for the *Azadirachta* genus. Figure 1 presents the chromatogram of hydroethanolic extract of the *A. indica* roots by UPLC-QToF-MS^E in according of elution order. Figure 2 showed the compounds tentatively identified.

The analysis of the spectra, through their specific fragments in MS^E, along with mechanistic analysis of literature data and the molecular formula, allowed us to provisionally identify nine molecules present in the hydroethanolic extract of neem roots: azadirachtin H, margosinolide, 6-deacetylnimbin, deacetylsalanine, nimbanal, nimbinene, salanol acetate, salanin and nimbin (Table 1 and 2).

Acute toxicity to D. rerio

After 48 h of toxicity tests of the neem hydroethanolic extract, mortality of test organisms was found to be less than 50%, even at the concentration of 2 mg mL^{-1}. These data indicated that the extract was not toxic to the evaluated organisms. In the literature, we did not found studies evaluating the toxicity of the hydroethanolic extract of neem roots in animal models.

However, ASHAFA et al., 2012, observed that the oral administration in rats of the ethanol extract from steamed bark of *A. indica* produced alteration in the biochemical parameters of the animals' organs. Acute and subacute toxicity studies performed by DORABABU et al., (2006), using aqueous extract from neem leaves indicate no mortality with a dose of 2.5 g kg^{-1} in mice and no significant changes in body weight or tissue appearance, or in the metabolism of this species. KANAGASANTHOSH et al., (2015), working with the ethanol extract from neem leaves, also identified no mortality in mice using doses up to 2 g kg^{-1}.

Antifungal activity

The tests revealed that the hydroalcoholic extract of neem has considerable antifungal activity against *T. rubrum* for both strains tested. However, it was inactive against *Candida* ssp for all strains evaluated (Table 2). In the literature, there are no reports of investigation of root activity in relation to the organisms evaluated in this study. However, in the discussion below, some studies carried out with neem that support this study are described.

MAHMOUD et al. (2011), evaluated the neem leaf hydroalcoholic extract against the fungi *Aspergillus flavus, A. fumigatus, A. niger, A. terreus, C. albicans* and *Microsporum gypseum* and found that the concentration of 5% (mass/volume) caused 44% inhibition *of A. flavus* and 20% of *C. albicans*, while the concentration of 15% caused absence mycelial growth. NATARAJAN et al. (2003), reported that the ethanol extracts from neem leaves had equal MIC and MFC values of 250 µg mL^{-1} against *T. rubrum*.

GOVINDACHARI et al. (1998), investigating the antifungal action of neem seed oil and its isolated compounds against *Drechslera oryzae, Fusarium oxysporum* and *Alternaria tenuis*, identified a reduction of the antifungal activity caused by the majority of isolated compounds, higher for salannim and azadiradione, while nimbin had low activity against most of the strains evaluated. The epoxyazadiradione in the pure form did not present any activity. The authors stated it is possible the main triterpenoids isolated has little or no antifungal activity, while in combination they presented excellent activity against all three fungi, suggesting potentiating/additive/synergetic effects.

For *T. rubrum*, the activity reported in the present study is similar to literature reports. For *Candida* ssp, the results found are not similar. The

Table 1 - UPLC-ESI-QTOF-MSE identification of compounds from hydroethanolic roots extract of *A. indica*.

Peak	Rt	$[M-H]^-$	$[M-H]^-$	Product	Empirical	ppm (error)	Putative Name	References
no.	min	Observed	Calculated	Ions (MS/MS)	Formula			
1	6.42	685.2479	685.2472	645.2620, 627.2390, 545.2062	$C_{33}H_{42}O_{14}Na$	1	Azadirachtin H	SHARMA, et al. 2003
2	7.03	457.2238	457.2226	425.1928, 421.1994, 407.1918, 389.1684	$C_{26}H_{33}O_7$	2.6	Nimbandiol	SANTOS et al. 2018
3	7.88	763.2377	763.2367	-	$C_{41}H_{40}O_{13}Na$	1.3	Unknow	-
4	7.03	479.2059	479.207	-	$C_{28}H_{31}O_7$	-2.3	Unknow	-
5	7.16	485.2172	485.2175	453.1903, 435.1869, 423.1822, 407.1708	$C_{27}H_{33}O_8$	-0.6	Margosinolide or Isomargosinolide	SIDDIQUI et al. 1986
6	7.52	275.1646	275.1647	187.0755, 137.0215	$C_{17}H_{23}O_3$	-0.4	Unknow	-
7	7.64	499.2321	499.2332	467.2049, 449.1920	$C_{28}H_{35}O_8$	-2.2	6-deacetylnimbin	HALDAR et al 2014
8	8.1	439.2101	439.2121	-	$C_{26}H_{31}O_6$	-4.6	Unknow	-
9	8.2	555.2962	555.2958	523.2700, 423.2242, 405.2168	$C_{32}H_{43}O_8$	0.7	Deacetylsalannin	HUANG et al. 1996 HALDAR et al. 2014
10	8.45	441.2275	441.2277	-	$C_{26}H_{33}O_6$	-0.5	Unknow	-
11	8.62	511.2323	511.2332	479.2018, 451.2162, 391.1883	$C_{29}H_{35}O_8$	-1.8	Nimbanal	HALDAR et al. 2014;
12	8.71	535.2267	535.2273	-	$C_{38}H_{31}O_3$	-1.1	Unknow	-
13	8.79	577.2382	577.2379	-	$C_{40}H_{33}O_4$	0.5	Unknow	-
14	8.88	503.2018	503.2011	-	$C_{37}H_{27}O_2$	1.4	Unknow	-
15	8.99	597.3071	597.3064	579.2917, 565.2866, 479.2468, 419.2227	$C_{34}H_{45}O_9$	1.2	Salannin	AARTHY et al. 2018
16	9.05	483.2384	483.2383	451.2119, 423.2156, 405.2066, 391.1945 373.1880,	$C_{28}H_{35}O_7$	0.2	Nimbinene	HALDAR et al. 2014
17	9.43	537.3013	537.3005	-	$C_{36}H_{41}O_4$	1.5	Unknow	-
18	9.49	301.2177	301.2168	-	$C_{20}H_{29}O_2$	3	Unknow	-
19	9.75	599.3207	599.322	581.3105, 567.2968, 497.2573, 437.2308	$C_{34}H_{47}O_9$	-2.2	Salannol acetate	HALDAR et al. 2014

*Rt: retention time; ppm (error): mass errors *(in* ppm*)*.

inactivity of the hydroalcoholic root extract can be related to the age of the plant, the time of collection, the soil at the collection site (variables that influence the production of secondary metabolites of the plant), as well as differences in the concentrations of bioactive compounds or the extractant solvent used, or even the absence of active substances found in other organs of the plant.

Antibacterial activity

The extract was not effective at any of the concentrations evaluated in inhibiting the growth

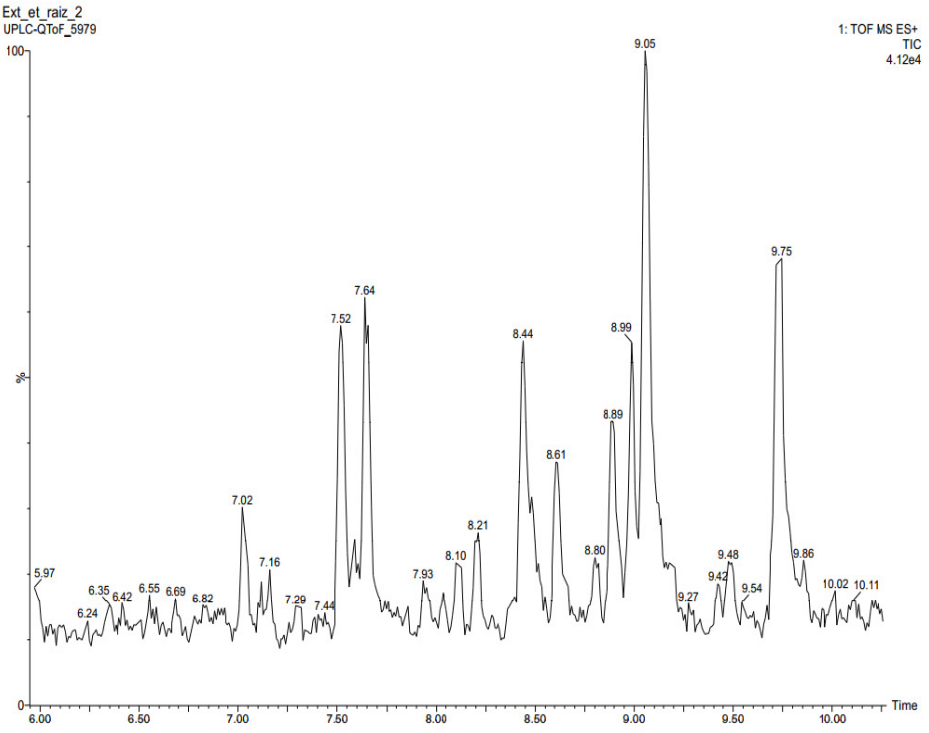

Figure 1 - Chromatogram of hydroethanolic extract of the *Azadirachta indica* roots by UPLC-QToF-MSE in according of elution order.

of colonies of *S. typhimurium* ATCC 14028 and *S. aureus* ATCC 27664.

Alves and collaborators, (2009), working with the hydroalcoholic extract of neem leaves against strains *S. aureus, S. thiphymurium, Bacillus subtilis, Aspergillus niger* and *Escherichia coli*, verified that the extract was effective only to inhibit the growth of *S. aureus* (MIC of 10^4 µg mL^{-1}). MEHROTRA et al., 2010, observed that the ethanol extract of neem leaves had strong activity against *Vibrio cholerae, Pseudomonas aeruginosa* and *S. aureus* (MIC of 0.25 µg $µL^{l-}$). MAHFUZUL et al., 2007, observed that the neem leaves extract presented higher antimicrobial activity against Gram-positive than Gram-negative bacteria. Among the microorganisms evaluated, the authors observed antibacterial activity against *Listeria monocytogenes, Vibrio parahaemolyticus, Bacillus cereus* and *S. aureus* (MIC of 4500 µg mL^{-1}), but no activity against *Escherichia coli* and *Salmonella enteritidis.*

The data in the literature only pertain to the activities of ethanol extracts of leaves, indicating moderate to strong activities against the evaluated organisms (*S. typhimurium* and *S. aureus*). However, it is necessary to highlight that the strains used in this study are different from those evaluated in previous studies and the substances were obtained from different organs of the plant, so the metabolic profile of the extract and the concentrations of the constituents are different. Although, the extract of neem root has been found to contain limonoid tetranortriterpenoids, include azadirachtin (a recognized bactericidal compound), it is possible that its low concentration in the extracts influenced the bioactivity against the evaluated bacteria.

Despite the high bioactive potential of neem, some municipal governments in Brazil have banned its cultivation (BOM JESUS DA LAPA (BA), 2021). This ban has occurred because some studies have shown that it can affect the reproductive activity of humans and animals, by promoting a decline of seminal parameters (volume and concentration of the ejaculate, motility and morphological changes of sperm), as well as through spermicidal action, vaginal contraceptive effect and interference in the estrous cycle of females in early embryonic development, promoting stillbirth (SILVA, 2010; URIBE-CLAVIJO et al., 2012; BRASIL, 2013).

Even so, due to its high bioactive potential, the plant would be an excellent source of income for

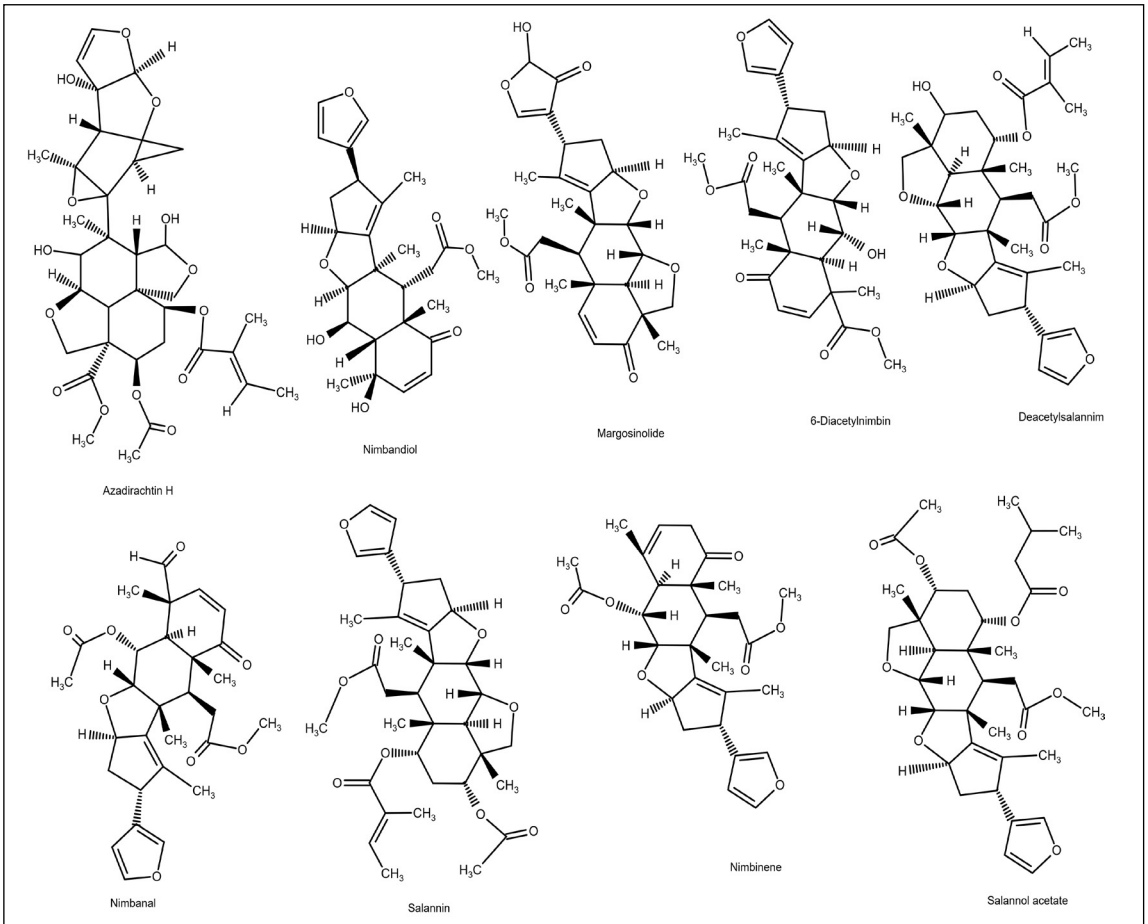

Figure 2 - Compounds tentatively identified in hydroethanolic extract of the *Azadirachta indica* roots by by UPLC-QTOF-MS^{E}.

rural populations in the semiarid region of Northeast Brazil, where it could be used a source to extract various natural products, since the species can be easily cultivated in this region. According to literature data, the plant adapts well to tropical and subtropical climates, with ideal cultivation temperatures of 21 to 32 °C, and the ability to withstand temperatures above 44 °C for short periods. It resists long periods of drought and annual rainfall between 400 and 800 mm. Finally, it can be cultivated in dry soils and soils poor in nutrients without harming its flowering (NEVES et al., 2003).

Table 2 - Antifungal activity of the hydroethanolic roots extract of *A. indica* against fungal test organism.

Strains	------Hydroethanolic extract of *A. indica*-----		----------Drug (µg mL^{-1})----------	
	MIC (µg mL^{-1})	MCF (µg mL^{-1})	Amphotericin B	Ketoconazole
C. tropicalis Labmic 0110	NI	NI	2	-
C. tropicalis Labmic 0112	NI	NI	1	-
C. albicans Labmic 0102	NI	NI	4	-
C. albicans Labmic 0104	NI	NI	2	-
T. rubrum Labmic 0210	39	78	-	1
T. rubrum Labmic 0204	78	156	-	1
T. rubrum Labmic 0209	78	156	-	1

*MIC: Minimum inhibitory concentration; MFC: Minimum fungicidal concentration; NI: no inhibition.

CONCLUSION

We identified nine compounds by UPLC-QTOF-MS^E. The extract had no acute toxicity to the zebrafish (*D. rerio*), but showed strong fungicidal activity against the *T. rubrum* strains evaluated. However, no activity was detected against *S. typhimurium*, S. *aureus*, *C. albicans* and *C. tropicalis*. Therefore, since neem adapts well to the climate of semiarid regions, the species has high bioactivity, as shown by the results obtained in this study and in literature reports, due to the consolidated uses of azadiractins as insecticides, it is possible to infer that the cultivation of this species could become a source of income for farmers in Northeast Brazil, which could use it to produce naturals fungicide and drug, as alternatives to conventional products, most of which cause microbiological resistance and/or are toxic to the environment, as well as being expensive.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

Certificate registered with the protocol 3915755/2018 by Ethics Committee in Animals Experimentation of the Universidade Estadual do Ceará (CEUA-UECE).

REFERENCES

AARTHY, T. Tracing the biosynthetic origin of limonoids and their functional groups through stable isotope labeling and inhibition in neem tree *(Azadirachta indica)* cell suspension. **BMC Plant Biology**, v.18, n.230, p.1-22, 2018. Available from: <https://doi.org/10.1186/s12870-018-1447-6>. Accessed: Jun. 15, 2020. doi: 10.1186/s12870-018-1447-6.

ALVES, P. D. et al. Chromatographic evaluation and antimicrobial activity of Neem (*Azadirachta indica* A. Juss., *Meliaceae*) leaves hydroalcoholic extracts. **Brazilian Journal of Pharmacognosy**, v.19, n.2 B, p.510–515, 2009. Available from: <https://doi.org/10.1590/S0102-695X2009000400001>. Accessed: May, 12, 2021. doi: 10.1590/S0102-695X2009000400001.

ANDRADE, V.; ROSSI, G. A. M. Dermatophytosis in company animals and its importance for Public Health – Literature Review. **Revista Brasileira de Higiene e Sanidade Animal: RBHSA**, ISSN-e 1981-2965, v.3, n.1, p.142–155, 2019. Available from: <https://repositorio.ufc.br/bitstream/riufc/55826/1/2019_art_vandrade.pdf>. Accessed: Jul. 15, 2020.

ASHAFA, A. O. T. et al. Toxicity profile of ethanolic extract of *Azadirachta indica* stem bark in male Wistar rats. **Asian Pacific Journal of Tropical Biomedicine**, p.811–817, 2012. Available from: <https://doi.org/10.1016/S2221-1691(12)60234-2>. Accessed: Jul. 2020. doi: 10.1016/S2221-1691(12)60234-2.

BALDA, A. C.et al. Retrospective survey of dermatophytosis in dogs and cats attended at the Serviço de Dermatologia da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo. **Acta Scientiae Veterinariae**, v.32, n.2, p.133-140, 27 jun. 2004. Available from: <https://doi.org/10.22456/1679-9216.16835>. Accessed: Jan. 15, 2021. doi: 10.22456/1679-9216.16835.

BERKOW, E. L.; LOCKHART, S. R. Fluconazole resistance *in Candida* species: a current perspective. **Infection and Drug Resistance**, v.10, p.237, 31 jul. 2017. Available from: <https://doi.org/10.2147/IDR.S118892>. Accessed: Jan. 15, 2021. doi: 10.2147/IDR.S118892.

BHOWMIK, D. et al. Herbal remedies of *Azadirachta indica* and its medicinal application debjit. **Journal of Chemical and Pharmaceutical Research**, v.3, n.2, p. 34–244, 2011. Available from: <https://www.jocpr.com/articles/herbal-remedies-of-azadirachta-indica-and-its-medicinal-application.pdf>. Accessed: Jul. 15, 2020. ISSN: 0975-7384.

BOM JARDIM DA LAPA (BAHIA). **LEI Nº 662 de 09 de junho de 2021**, 2021.

LEE, A. S. et al. Methicillin-resistant *Staphylococcus aureus*. **Nature Publishing Group**, v.4, p.1-23, 2018. Available from: <https://doi.org/10.1038/nrdp.2018.33>. Accessed: Jun. 12, 2021. doi: 10.1038/nrdp.2018.33.

BRASIL, R. B. Botanical aspects, traditional uses and potentials of *Azadirachta indica* (Neem). **Enciclopédia Biosfera**, v.9, n.17, p.3252–3268, 2013. Available from: <https://www.conhecer.org.br/enciclop/2013b/MULTIDISCIPLINAR/Aspectos.pdf>. Accessed: Jul. 15, 2020.

CECONI, J. E. et al. Evaluation of pharmacological treatments for dermatophytoses in pet animals. **Pubvet**, v.12, n.4, p.1–10, abr. 2018. Available from: <https://doi.org/10.22256/pubvet.v12n4a74.1-10>. Accessed: Dec. 16, 2021. doi: 10.22256/pubvet.v12n4a74.1-10.

CLINICAL AND LABORATORY STANDARDS INSTITUTE (CLSI). **Reference Method for Broth Dilution**. v.28, n.14, 2008.

COSTA, J. V. T. A. et al. Use of oil and aqueous extract of Neem seeds, azadirachtin and acephate to control cowpea black aphid. **Pesquisa Agropecuaria Tropical**, v.40, n.2, p.238-

241, 2010. Available from: <https://revistas.ufg.br/pat/article/view/3564/6904>. Accessed: May, 16, 2020. e-ISSN 1983-4063.

CUNICO, M. M. et al. Study *in vivo* of antifungical activity *Ofottonia martiana Miq*., *piperaceae*. **Visão Acadêmica**, v. 4, n. 2, p. 72–82, 31 dez. 2003. Available from: <https://www.researchgate.net/profile/Celso-Auer/publication/287431350>. Accessed: jun 16, 2020.

DALLAQUA, B. et al. Azadirachta indica treatment on the congenital malformations of fetuses from rats. **Journal of Ethnopharmacology**, v.150, n.3, p.1109-1113, 2013. Available from: <https://doi.org/10.1016/j.jep.2013.10.046>. Accessed: Jun. 15, 2020. doi: 10.1590/S0100-736X2008000600006.

DAVID, P. E. et al. HIV-1 infection inhibition by neem (Azadirachta indica A. Juss.) leaf extracts and Azadirachtin. **Indian Journal of Traditional Knowledge**, v.16, n.3, p.437–441, 2017. Available from:____<https://www.researchgate.net/profile/David-Pedroza-2/publication/343218681>. Accessed: Jan. 12, 2021.

DELEITO, C. S. R.; BORJA, G. E. M.; Neem (*Azadirachta indica*): An alternative for controlling flies associated with animal breeding. **Pesquisa Veterinaria Brasileira**, v.28, n.6, p.293–298, 2008. Available from: <https://doi.org/10.1590/S0100-736X2008000600006>. Accessed: Dec. 10, 2020. doi: 10.1590/S0100-736X2008000600006.

DORABABU, M.; et al. Effect of aqueous extract of neem (*Azadirachta indica*) leaves on offensive and diffensive gastric mucosal factors in rats. **Indian Journal of Physiology and Pharmacology**, v.50, n.3, p.241–249, 2006. Available from: <https://www.researchgate.net/profile/Madhura-Babu/publication/6606560>. Accessed: Dec. 10, 2020.

FONTENELLE, R. O. et al. Chemical composition, toxicological aspects and antifungal activity of essential oil from *Lippia sidoides* Cham. **J Antimicrob Chemother**. v.59, n.5p. 934-40, 2007. Available from: <https://doi.org/10.1093/jac/dkm066>. Accessed: Jul. 10, 2021. doi: 10.1093/jac/dkm066.

GOVINDACHARI, T. R. et al. Identification of antifungal compounds from the seed oil of *Azadirachta indica*. **Phytoparasitica**, v.26, n.2, p.109–116, 1998. Available from: <https://link.springer.com/article/10.1007/BF02980677>. Accessed: Jun. 10, 2020.

GUPTA, S. C. et al. Neem (*Azadirachta indica*): An indian traditional panacea with modern molecular basis. **Phytomedicine**, v.34, p.14–20, 2017. Available from: <https://doi.org/10.1016/j.phymed.2017.07.001>. Accessed: mar. 12, 2021. doi: 10.1016/j.phymed.2017.07.001.

HALDAR, S. et al. Expedient preparative isolation and tandem mass spectrometric characterization of C-seco triterpenoids from Neem oil. **Journal of Chromatography A**, p.1-14, 2014. Available from: <https://doi.org/10.1016/j.chroma.2014.09.006>. Accessed: Jun. 20, 2020. doi: 10.1016/j.chroma.2014.09.006.

HUANG, C. R. et al. Limonoids from *Melia azedarach*. **Phytochemistry**, v.43, n.3, 1996. Available from: <https://doi.org/10.1016/0031-9422(96)00353-6>. Accessed: Mar. 20, 2020. doi: 10.1016/0031-9422(96)00353-6.

KANAGASANTHOSH, K. et al. Evaluation of acute toxicity, anti-inflammatory activity and phytochemical screening of ethanolic extract of *Azadirachta indica* leaves. **Int. J. Res. Dev. Pharm. L. Sci. International Journal of Research and Development in Pharmacy and Life Sciences**, v.4, n.5, p.1737–1742, 2015. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3609225/>. Accessed: Mar. 12, 2021. doi: 10.1016/S2221-1691(12)60234-2. Accessed: Mar. 20, 2021. ISSN: 2349–8528.

KUMAR, R. et al. Bioactive constituents of neem. **Synthesis of Medicinal Agents from Plants**. Chapter 4, p.75-103, 2018. Available from: <https://doi.org/10.1016/B978-0-08-102071-5.00004-0>. Accessed: Mar. 12, 2021. doi: 10.1016/B978-0-08-102071-5.00004-0.

KUMAR, V. S.; NAVARATNAM, V. Neem (*Azadirachta indica*): Prehistory to contemporary medicinal uses to humankind. **Asian Pacific Journal of Tropical Biomedicine**, v.3, n.7, p.505-514, 2013. Available from: <https://doi.org/10.1016/S2221-1691(13)60105-7>. Accessed: Mar. 12, 2021. doi: 10.1016/S2221-1691(13)60105-7.

KURIMOTO, S. I. et al. Triterpenoids from the fruits of *Azadirachta indica (Meliaceae)*. **Fitoterapia**, v.92, p.200-205, 2014. Available from: <https://doi.org/10.1016/j.fitote.2013.11.004>. Accessed: Jun. 12, 2020. doi: 10.1016/j.fitote.2013.11.004.

KUSHIDA, T.; WATANABE, S. Canine ringworm caused by *Trichophyton rubrum*; probable transmission from man to animal. **Sabouraudia**, v.13 p.1, n.1, p.30-2, 1975. Available from: <https://doi.org/10.1080/00362177585190051>. Accessed: Nov. 12, 2020. doi: 10.1080/00362177585190051.

MAHFUZ HOQUE, M. D. et al. Antibacterial Activity of Guava (*Psidium guajava* L.) and Neem (*Azadirachta indica* A. Juss.) Extracts Against Foodborne Pathogens and Spoilage Bacteria. **Foodborne Pathogens and Disease**, v.4, n.4, p.481–488, 2007. Available from: <https://doi.org/10.1089/fpd.2007.0040>. Accessed: Nov. 12, 2020. doi: 10.1089/fpd.2007.0040.

MAHMOUD, D. A. et al. Antifungal activity of different neem leaf extracts and the nimonol against some important human pathogens. **Brazilian Journal of Microbiology**, v.42, n.3, p.1007–1016, 2011. Available from: <https://doi.org/10.1590/S1517-83822011000300021>. Accessed: Jun. 15, 2021. doi: 10.1590/S1517-83822011000300021.

MEHROTRA, S. et al. Comparative antimicrobial activities of Neem, Amla, Aloe, Assam Tea and Clove extracts against *Vibrio cholerae, Staphylococcus aureus* and *Pseudomonas aeruginosa*. **Journal of Medicinal Plants Research**, v.4, n.23, p.2473–2478, 2010. Available from: <https://www.researchgate.net/profile/Shoma-Nandi/publication/267973020>. Accessed: Nov. 15, 2020. ISSN 1996-0875 ©2010.

MORDUE(LUNTZ), A. J.; NISBET, A. J. Azadirachtin from the neem tree *Azadirachta indica*: its action against insects. **Anais da Sociedade Entomológica do Brasil**, v.29, n.4, p.615–632, 2000. Available from: <https://doi.org/10.1590/S0301-80592000000400001>. Accessed: Jun. 16, 2021. doi: 10.1590/S0301-80592000000400001.

MORIELLO, K. A. et al. Diagnosis and treatment of dermatophytosis in dogs and cats.: Clinical Consensus Guidelines of the World Association for Veterinary Dermatology. **Veterinary dermatology**, v.28, n.3, p.266–268, 1 jun. 2017. Available from: <https://doi.org/10.1111/vde.12440>. Accessed: Jun. 12, 2020. doi: 10.1111/vde.12440.

NATARAJAN, V. et al. Effect of *Azadirachta indica* (neem) on the growth pattern of dermatophytes. **Indian journal of medical mic robiology**, v.21, n.2, p. 98–101, 2003. Available from: <https://doi.org/10.1016/S0255-0857(21)03129-7>. Accessed: Jul. 12, 2020. doi: 10.1016/S0255-0857(21)03129-7.

NEVES, B. P. et al. Cultivo e utilização do nim indiano. **Embrapa**. Goiania, 2003. Available from: <https://www.embrapa.br/busca-de-publicacoes/-/publicacao/212487/cultivo-e-utilizacao-do-nim-indiano>. Accessed: Dec. 05, 2021.

PASCOLI, M. et al. Neem oil based nanopesticide as an environmentally-friendly formulation for applications in sustainable agriculture: An ecotoxicological perspective. **Science of the Total Environment**, v.677, p.57–67, 2019. Available from: <https://doi.org/10.1016/j.scitotenv.2019.04.345>. Accessed: Jan. 13, 2021. doi: 10.1016/j.scitotenv.2019.04.345.

PFALLER, M. A. et al. Twenty years of the sentry antifungal surveillance program: results for candida species from 1997-2016. **OFID**, v.2019, n.6, p.79, 1997. Available from: <https://doi.org/10.1093/ofid/ofy358>. Accessed: Jun. 14, 2020. doi: 10.1093/ofid/ofy358.

REIS, L. B. et al. Dermatofitose pustular em um felino por *Tricophyton rubrum*: relato de caso. **Pubvet**, v.14, n.1, p.1-5, 2020. Available from: <http://www.pubvet.com.br/artigo/6709/dermatofitose-pustular-em-um-felino-por-tricophyton-rubrum-relato-de-caso>. Accessed: Jan. 12, 2021. doi: 10.31533/pubvet.v14n1a487.1-5.

SANTOS, A. M. P. et al. Virulence Factors in *Salmonella Typhimurium:* The Sagacity of a Bacterium. **Current Microbiology**, v.76, p.762–773, 2019. Available from: <https://link.springer.com/article/10.1007/s00284-018-1510-4>. Accessed: Jun, 16, 2020. doi: 0.1007/s00284-018-1510-4.

SHAAPAN, R. M. et al. *Myrtus communis* Essential Oil; anti-parasitic effects and induction of the innate immune system in mice with toxoplasma gondii infection. **Molecules**, v.26, n.4, p.1-10, 2021. Available from: <https://doi.org/10.3390/molecules26040819>. Accessed: Dec. 16, 2021. doi: 10.3390/molecules26040819.

SIDDIQUI S. et al. Margosinolide at isomargosinolide, two new tetranortriterpenoids from *Azadirachta indica A*. Juss (*Meliaceae*). **Tetrahedron**, v.42, n.17, p.4849-4856, 1986. Available from: <https://doi.org/10.1016/S0040-4020(01)82066-4>. Accessed: Jun. 16, 2020. doi: 10.1016/S0040-4020(01)82066-4.

SILVA, V. C. L. **Avaliação da toxicidade reprodutiva de ratas wistar submetidas à ingestão do extrato etanólico das folhas de nim (Azadirachta indica A. Juss)**. [s.l.] Universidade Federal Rural de Pernambuco, 2010. Available from: <http://www.tede2.ufrpe.br:8080/tede2/handle/tede2/5879>. Accessed: jul. 12, 2020.

SIVACHIDAMBARAMA, M. et al. A novel synthesis protocol for Co3O4 nanocatalysts and their catalytic applications. **RSC Advances**, v.7, n.62, p.38861-38870, 2017. Available from: <https://doi.org/10.1039/C7RA06996K>. Accessed: Jan. 16, 2021. doi: 10.1039/C7RA06996K.

TURNER, N. A. et al. Methicillin-resistant *Staphylococcus aureus*: an overview of basic and clinical research. **Nature Reviews Microbiology 2019 17:4**, v.17, n.4, p.203-218, 2019. Available from <https://journal.ohrm.bba.md/index.php/journal-ohrm-bba-md/article/view/191>. Accessed: Jun.14, 2020. doi: 10.1038/s41579-018-0147-4.

URIBE-CLAVIJO, M. et al. Espermicidas: Una Alternativa de Anticoncepción para Considerar Spermicides: A Contraception Alternative to Consider. **Revista Tecno Lógicas**, n.28, p.129–145, 2012. Available from: <http://www.scielo.org.co/pdf/teclo/n28/n28a08.pdf>. Accessed: Jan, 20, 2021. ISSN: 0123-7799.

ZHU, J. et al. A new cytotoxic salannin-class limonoid alkaloid from seeds of *Azadirachta indica* A. Juss. **Chinese Chemical Letters**, v.29, n.8, p.1261–1263, 2017. Available from: <https://doi.org/10.1016/j.cclet.2017.11.042>. Accessed: Jan. 12, 2021. doi: 10.1016/j.cclet.2017.11.042.