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Antibacterial Activity of Methanolic Extracts of *Zanthoxylum zanthoxyloides* (Lam.) B. Zepernich and Timler (Rutaceae) Justifying its Use in Traditional Medicine to Fight against Infection

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ABSTRACT

The objective of this study was to evaluate the antimicrobial activity of extracts of different parts of Zanthoxylum. zanthoxyloides on a large panel of 36 bacteria. The MICs were determined by successive dilution of the extracts in solid MHA medium. The inhibitory activity of the extracts was measured over a concentration range of 78 to 1250 mg/L. The best activity on a broad spectrum of bacteria was obtained with the root extract; it was active on 20 strains with MIC values ranging from 78 to 625 mg/L. The highest antimicrobial properties (MIC=78 mg/L) were observed against Staphylococcus pettenkoferi T28-2, Dermabacter hominis T47A7, Streptococcus pyogenes 13240 and Pseudomonas aeruginosa T4-1. Fruit, leaf and bark extract showed growth inhibition of two strains: Staphylococcus pettenkoferi T28-2 and Dermabacter hominis T47A7. These results demonstrate the presence of antimicrobial molecules in the roots of Z. zanthoxyloides and corroborate their use in traditional medicine to treat infectious diseases.

Keywords: *Zanthoxylum zanthoxyloides*; Methanolic extracts; Roots; Antibacterial activity

INTRODUCTION

Since their appearance, antibiotics have remained the preferred way to fight against bacterial infections. However, because of their anarchic, inadequate and abusive use in human and veterinary health, we are witnessing the emergence of multidrug-resistant bacteria. In 2011, the WHO (World Health Organization) called for increased research on new drugs as antibiotic resistance increases dramatically, but only a few new molecules are being developed [1,2]. Thus, the lack of real prospects for the discovery of new antibiotics in the years to come, led us to study the effectiveness of plants with therapeutic properties to isolate active ingredients.

Z. zanthoxyloides (Rutaceae) is an aromatic and medicinal plant widely used in traditional medicine in the treatment of abdominal and dental problems, sickle cell disease, leukoderma, asthma, fever and dyspepsia [3,4]. The therapeutic potentialities of *Z. zanthoxyloides* extracts have been reported in several scientific works; they have insecticidal [5], anthelmintic [6], antiplasmodial [7,8], vasodilator [9], antifalcemic [10], cytotoxic [11,12], anti-inflammatory [13] and antibacterial [14,15] properties.

The vast majority of these studies focus on the antibacterial activities of the roots [14-20]. extracts of *Z. zanthoxyloides* showed activity against Gram-positive bacteria (*Enterococcus faecalis*, *Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus auricularis*, *Streptococcus pyogenes*, *Streptococcus mutans*, *Bacillus subtilis*, *Streptococcus spp*, *Lactobacillus brevis*, *Lactobacillus plantarum*) and Gram negative (*Porphyromonas gingivalis*, *Porphyromonas nigrescens*, *Prevotella intermedia*, *Haemophilus* spp, *Escherichia coli*, *Neisseria spp*, *Proteus mirabilis*, *Proteus vulgaris*), but generally very few strains of each species were studied.

Thus, the objective of our study was to evaluate the antibacterial activity of the various extracts (leaves, fruits, stems, barks and roots) of *Z. zanthoxyloides* on 36 bacterial strains.

MATERIAL AND METHODS

Plant Material

The fruit, leaf, stem, root and bark samples (Figure 1) of *Z. zanthoxyloides* were harvested in May 2015 (fruit ripening period) from only tree, growing wild in one Senegalese locality, Kafountine (12°56′5.49926″ N, 16°44′45.28315″ W). The botanical identification of the plant material was performed by Dr. William Diatta from the Department of botanical and pharmacognosy of University Cheikh Anta Diop of Dakar (Senegal).

Figure 1. Fruits (1) leaves and stems (2) barks (3) and roots (4) of *Z. zanthoxyloides*

Plant Extracts

Each plant organ (fruits, leaves, roots, stems and barks) has been extracted separately. Plant samples were air dried for a period of four weeks at ambient temperature. The plant material was powdered with an average particle size of 0.2 mm using a blade miller (Polymix PX-MFC 90D, KINEMATICA AG, Luzern, Switzerland). For each sample, 50 gms of powder were extracted with 3 × 200 mL of methanol over 48 hrs, each time, at room temperature under magnetic stirring. The three solutions were combined, filtered through filter paper (PRATDUMAS, Couze-St-Front, France) and evaporated to dryness using a rotary evaporator (Laborota 4000, Heidolph, Schwabach, Germany). The extract yields (w/w, calculated on a dry weight plant) were 27.8%, 16.3%, 20.6%, 5.2%, and 14.2% for fruits, leaves, roots, stems and barks, respectively. In order to remove the chlorophyll from the methanolic leaf extract, 2.5 gms of extract was dissolved in 100 mL of methanol and extracted four times with 150 mL of hexane. The yield of the residual methanol extract was 70%.

Microbial Strains

The microorganisms used in these extract studies include strains from the bacteriology laboratory collection (INSERM U995), recently obtained by isolating clinical specimens (essentially diabetic foot wounds) and reflecting the antibiotic resistance encountered today in hospitals and reference strains of the American Type Culture Collection (ATCC) often isolated a long time ago, but useful for inter-laboratory comparison. Antimicrobial assays were performed in vitro culture on 36 microbial strains including 32 Gram-positive bacteria and 4 Gram-negative bacteria capable of growing in an aerobic Mueller Hinton agar medium (MHA). The minimum inhibitory concentrations (MICs) of the extracts were determined using the solid-state dilution method according to CLSI standards [21]. The concentrations analyzed ranged from 78 to 1250 mg/L corresponding to five half-fold dilutions (1250, 625, 312, 156 and 78 mg/L). The Petri dishes (solvent controls and extracts) were inoculated with different bacterial suspensions (10^6 CFU/mL, obtained by dilution of a 24 hrs culture in MHA) using a Steers replicator and were incubated at 37° C for 24 hours. MIC was defined as the lowest concentration of extract without visible bacterial growth after incubation. Extracts with a MIC below 100 mg/L have a good antibacterial activity. Between 100 and 500 mg/L, we speak of a moderate antibacterial activity, between 500 and 1000 mg/L, the antibacterial activity is called weak and finally the extract is considered as inactive for a MIC greater than 1000 mg/L [22].

RESULTS AND DISCUSSION

The antimicrobial activity of *Z. zanthoxyloides* extracts was evaluated against 32 Gram-positive and 4 Gram-negative bacteria. The results of the MIC values shown in Table 1 indicate that the extracts of the various organs have varying antibacterial activities depending on the strains tested.

The inhibitory activity of the extracts was measured over a concentration range of 78 to 1250 mg/L. The best activity on a broad spectrum of bacteria was obtained with the root extract; it was active on 20 strains with MIC values ranging from 78 to 625 mg/L. Although the extracts are inactive on *Staphylococcus aureus* (11 strains tested), the properties against the other Gram-positive strains are remarkable. These strains have attracted interest in recent years because they are also endowed with many virulence factors. The strongest antimicrobial properties (MIC=78 mg/mL) were observed against *Staphylococcus pettenkoferi* T28-2, *Dermabacter hominis* T47A7, *Streptococcus* *pyogenes* 13240 and *Pseudomonas aeruginosa* T4-1. Fruit, leaf, stem and bark extracts showed growth inhibition of two strains of *Staphylococcus pettenkoferi* T28-2 and *Dermabacter hominis* T47A7.

Table 1. Antibacterial activities of *Z. zanthoxyloides* extracts

Strains		MIC of the different extracts (mg/mL)				
		Fruits	**Leaves**	**Roots**	**Stems**	**Barks**
Bacteria Gram (+)	*Staphylococcus aureus* T25-10	-	-	625	-	-
	Staphylococcus aureus T28-1	-	-	-	-	-
	Staphylococcus aureus 8143	-	-	-	-	-
	Staphylococcus aureus 8146	-	-	625	-	-
	Staphylococcus aureus 8148	-	-	625	-	-
	Staphylococcus aureus 8241	-	-	625	-	-
	Staphylococcus aureus T6-1	-	-	-	-	-
	Staphylococcus aureus T2-1	-	-	-	-	-
	Staphylococcus aureus T1-1	-	-	-	-	-
	Staphylococcus aureus T30-6	-	-	-	-	-
	Staphylococcus aureus T26A4	-	-	625	-	-
	Staphylococcus epidermidis T15-1	-	-	-	-	-
	Staphylococcus epidermidis T19A1	-	-	-	-	-
	Staphylococcus capitis T21A3	-	-	312	-	-
	Staphylococcus capitis T29A2	-	-	312	-	-
	Staphylococcus pettenkoferi T28-2	156	625	78	-	312
	Staphylococcus pettenkoferi T3-3	-	-	625	-	-
	Staphylococcus warneri T12A12	-	-	-	-	-
	Staphylococcus saprophyticus 8237	-	-	625	-	-
	Staphylococcus lugdunensis T36A1	-	-	312	-	-
	Staphylococcus lugdunensis T47B2	-	-	312	-	-
	Corynebacterium striatum T40A3	-	-	312	-	-
	Corynebacterium striatum T46C1	-	-	312	-	-
	Dermabacter hominis T47A7	156	312	78	625	78
	Dermabacter hominis T49B5	-	-	312	-	-
	Streptococcus agalactiae T25-7	-	-	312	-	-
	Streptococcus agalactiae T53A4	-	-	312	-	-
	Streptococcus pyogenes 13240	-	-	78	625	-
	Streptococcus pyogenes 13241	-	-	-	-	-
	Gemella haemolysans T46B5	-	-	-	-	-
	Enterococcus faecalis T37B1	-	-	-	-	-
	Enterococcus faecalis T47A16	-	-	-	-	-
Bacteria Gram (-)	*Escherichia coli* ATCC 25922	-	-	-	-	-
	Escherichia coli T20A2	-	-	-	-	-
	Pseudomonas aeruginosa ATCC 27583	-	-	-	-	-
	Pseudomonas aeruginosa T4-1	-	-	78	-	-

These results demonstrate the presence of antimicrobial molecules in the roots of *Z. zanthoxyloides* and corroborate their use in traditional medicine to treat infectious diseases. The antibacterial activity is mainly focused on deep skin infections that do not involve *S. aureus*. Moreover, our observations are in line with the conclusions of articles on root extracts; these have exhibited in particular an activity against a variety of Gram-positive and Gram-negative bacteria. These antimicrobial properties have been shown to be due to the presence of alkaloids in the roots of *Z. zanthoxyloides*, especially to the aporphine-type molecules. (tembetarine, berberine, magnoflorine), with furoquinolines (8-methoxydictamine, skimmianine, 3-dimethylallyl-4-méthoxy-2-quinolone) and benzophenanthridine (fagaronine, dihydroavicin, chelerythrine and canthin-6-one) [23-25]. Other molecules with antibacterial activities have also been reported by Chaaib et al. (2003): four phenylpropane derivatives (dihydrocuspidiol, cuspidiol, 4’-O-(3’’-methylbut-2’’-enyloxy)-3-phenylpropanol and sesamin) and an alcamide (pellitorine) [21-24]. The analysis of these pure products will be continued, but often the action is synergistic, so the crude extract is often more active than its pure compounds.

In this work, we analyzed the ability of root extracts of *Z. zanthoxyloides* to inhibit the growth of a panel of strains isolated mainly from deep skin infections. In view of the results, it would be interesting now to adapt to other types of bacteria involved in other pathologies. Preliminary work based on the measurement of minimum inhibitory concentrations indicates interesting effects on periodontopathogens such as the anaerobic bacteria *Porphyromonas gingivalis* and *P. nigrescens* [26-28]. It would also be useful to test a panel of strains associated with these pathologies by determining the MICs.

But substances of plant origin which are often lipophilic can also have an effect on certain virulence factors (without inhibition of growth) such as biofilm formation. A biofilm is characterized by a dense extracellular matrix that forms around bacteria attached to a surface. This matrix prevents the spread of antibiotics making these infections so difficult to treat [29-31]. It is estimated today that 60% of infections are in the form of a biofilm [31]. The anti-biofilm action of a substance can disintegrate this matrix without damaging the bacteria, but can make the action of antibiotics effective again.

CONCLUSION

This study described, for the first time, the antimicrobial activity of extracts of the various organs of *Z. zanthoxyloides* on a large panel of bacteria using a reproducible, standardized method recommended by CLSI. The best activity on a broad spectrum of bacteria was obtained with the root extract. Thus, the continuation of our study will be to refine the action of crude extracts and purified products by expanding the bacterial spectrum and seeking action on certain virulence factors.

REFERENCES

1. A Abedini; V Roumy; S Mahieux; M Biabiany; A Standaert-Vitse; C Rivière. *Evid-Based Complement Altern Med.* **2013**.
2. AC Abreu; AJ McBain; M Simoes. *Nat Prod Rep*. **2012**, 29(9): 1007-1021.
3. Y Tine; A Diop; W Diatta; J-M Desjobert; CSB Boye; J Costa. *Chem Biodivers*. **2017**, 14(1).
4. Y Tine; F Renucci; J Costa; A Wélé; J Paolini. *Molecules*. **2017**, 22(1): 174.
5. AA Denloye; WA Makanjuola; O Ajelara; OJ Akinlaye; RA Olowu; OA Lawal. *Julius-Kühn-Arch*. **2010**,

425: 833-839.

6. BB Barnabas; A Mann; TS Ogunrinola; PE Anyanwu. *J Appl Res Nat Prod*. **2010,** 3(4): 1-4.
7. A Gansané; S Sanon; LP Ouattara; A Traoré; S Hutter; E Ollivier. *Parasitol Res*. **2010**, 106(2): 335-340.
8. Kassim OO; Loyevsky M; Elliott B; Geall A; Amonoo H; Gordeuk VR. *Antimicrob Agents Chemother*. **2005**, 49(1): 264-248.
9. SO Zahoui; NG Zirihi; YT Soro; F Traore. *Phytothérapie*. **2010**, 8: 359-369.
10. EA Sofowora; WA Isaac-Sodeye; LO Ogunkoya. *Lloydia*. **1975**, 38(2): 169-171.
11. NA Vyry Wouatsa; LN Misra; R Venkatesh Kumar; MP Darokar; F Tchoumbougnang. *Nat Prod Res*. **2013**, 27(21): 1994-1998.
12. Y Ogunbolude; M Ibrahim; OO Elekofehinti; A Adeniran; AO Abolaji; JBT Rocha; *J Intercult Ethnopharmacol*. **2014**, 3(4): 167-172.
13. W Diatta; GY Sy; CI Manga; K Diatta; A Fall; E Bassene. *Int J Biol Chem Sci*. **2014**, 8(1): 128-133.
14. Ynalvez RA; Cardenas C; Addo JK; Adukpo GE; Dadson BA; Addo-Mensa A. *Res J Med Plant*. **2012**, 6(2): 149-159.
15. M Adefisoye; K AkoNai; M Bisi Johnson. *J Intercult Ethnopharmacol*. **2012**, 1(1): 1.
16. O Taiwo; HX Xu; SF Lee. *Phytother Res*. **1999,** 13: 675-659.
17. A Adekunle; K Odukoya. *Ethnobot Leafl*. **2006**, 2006(1).
18. AO Adebiyi; T Koekemoer; AP Adebiyi; N Smith; E Baxter; RJ Naude; *Pharm Biol*. **2009**, 47(4): 320-327.
19. LO Orafidiya; EO Akinkunmi; FO Oginni; A Oluwamakin. *Niger J Nat Prod Med*. **2010**, 14: 21-26.
20. EO Oshomoh; M Idu. *Int J Med Aromat Plants*. **2012**, 2(3): 411-419.
21. MA Wikler. *CLSI NCCLS*. **2006**, 26: M7-A7.
22. A Landoulsi; V Roumy; N Duhal; FH Skhiri; C Rivière; S Sahpaz. *Chem Biodivers*. **2016**,13(12): 1720-1729.
23. OO Odebiyi; EA Sofowora. *Planta Med*. **1979**, 36(07): 204-207.
24. Mohammed S; Mohammed A. *Pak Med Assoc*. **1987**, 31: 4-8.
25. SK Adesina. *Afr J Trad CAM*. **2005**, 2(3): 282-301.
26. R Teanpaisan; S Senapong; J Puripattanavong. *J Pharm Res*. **2014**; 13(7): 1149-1155.
27. DKR Bardají; EB Reis; TCT Medeiros; R Lucarini; AEM Crotti; CHG Martins. *Nat Prod Res*. **2016**, 30(10): 1178-1181.
28. DKR Bardají; JJM da Silva; TC Bianchi; D de Souza Eugênio; PF de Oliveira; LF Leandro. **2016**, 40: 18-27.
29. G Hughes; MA Webber. *Br J Pharmacol*. **2017**, 174(14): 2237-2346.
30. TR Garrett; M Bhakoo; Z Zhang. *Prog Nat Sci*. **2008**, 18(9): 1049-1056.
31. JP Pintucci; S Corno; M Garotta. *Eur Rev Med Pharmacol Sci*. **2010**, 14(8): 683-690.