IJPSR (2022), Volume 13, Issue 1 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 30 March 2021; received in revised form, 25 May 2021; accepted, 02 June 2021; published 01 January 2022

PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF EXTRACTS OF *AZADIRACHTA INDICA* A. JUSS (MELIACEAE) (CÔTE D'IVOIRE)

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Keywords:

Azadirachta indica, Extract, Phytochemical screening, Antioxidant activity, Antibacterial activity

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ABSTRACT: The purposes assigned to this work are the phytochemical screening of certain phytoconstituents, the estimation of the antioxidant and antibacterial activities of extracts of *Azadirachta indica* A. Juss. The selective extracts obtained from the aqueous decocts were used to establish the chromatographic profile of the various extracts. As for the crude aqueous extracts, they were used to carry out the tests by color reactions to evaluate their antioxidant potential as well as their antibacterial activity against 12 strains. Phytochemical screening revealed the existence of a few bio-important phytoconstituents. The median effective reduction concentration (CR50 = 0.10 mg / mL) demonstrated the antioxidant efficacy of trunk bark decocted (ET) vis-à-vis DPPH, compared with vitamin C (CR50 = 0.006 mg / mL), the reference antioxidant. Also, the extracts, in particular ET, exhibited almost bactericidal activity against the strains tested with the exception of *Pseudomonas aeruginosa* 469 UB / 20 CNRa. Both the antioxidant and the antibacterial activity of the plant vary depending on the type of extract, organ and bacterial strain.

INTRODUCTION: Plants are the natural reservoir par excellence of molecules of high therapeutic and pharmacological value. This is why much scientific work in the field of plant chemistry is being undertaken to discover new molecular structures for human well-being. *Azadirachta indica* A. Juss (or margosier), commonly known as "neem", is a tree native to India 1 of the botanical family Meliaceae. It is known there under the names "village pharmacy" or "tree of freedom" thanks to the various applications.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(1).417-26

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(1).417-26

Indeed, all its parts (bark, fruits, seeds, leaves) exhibit insect repellent, antibacterial, antifungal, antiplasmodial, antioxidant, anticancer, antiviral properties, *etc.* [2-8]. Today, the plant (6-15 m in height) is cultivated in tropical and semi-tropical regions as an orange tree, and a decoction of the leaves and stem bark is a febrifuge5.

According to Kausik *et al.*, [9] chemical investigations on this plant species indicate that at least 135 compounds (coumarins, flavonoids, polyphenols, tannins, triterpenes, *etc*.) have been isolated from its different parts. In Côte d'Ivoire, *A. indica* responds to local names: djindé baté, djindé gni (in Akyé); djaba baka, djaba ouaka (in Agni, Baoulé) [5]. The leaves, trunk, and root bark of *A. indica* are used in traditional medicine to treat malaria. However, this plant seems to be the

miracle panacea that cures all ailments, which is why it has sparked a sudden enthusiasm among the Ivorian populations in the search for a response against the pathogen (Sars-CoV2), responsible for COVID- 19. Very little scientific data on *A. indica* growing in Côte d'Ivoire are reported in the literature. The objective of the present study is to determine the prior phytochemical composition of extracts from the leaves, root bark and trunk of the plant and to evaluate their antioxidant and antibacterial activities.

MATERIAL:

Plant Material: The plant material consists of the leaves, bark of the trunk, and the root of A. indica. It was collected in July 2020 at Nangui Abrogoua University located in the municipality of Abobo (5 ° 23 ′ 21.145 ″ north, 4 ° 00 ′ 59.236 ″ west) in Abidjan (Côte d'Ivoire). The identification was made at the National Floristic Center of Abidjan, and specimens are deposited in the bio-organic chemistry and natural substances laboratory at the Fundamental and Applied Sciences Training and Research Unit (UFR-SFA). The plant material was then cut up, cleaned, left to dry under air conditioning (18 °C) for 40 days, and then pulverized. The powders obtained were conditioned for the various analyzes.

Bacterial Strains: The bacterial strains were provided by the biobank of the Institut Pasteur de Côte d'Ivoire (IPCI):

Fermentative Enterobacteriaceae (ferments glucose):

• *Escherichia coli* 466 TR/20 CNRa: Resistant producer of extended spectrum betalactamase (ESBL), resistant to fluoroquinolones (FQPS02).

• *Escherichia coli* 470 UB/20 CNRa: FQPS02, Cephalosporinase hyperproduction (HPCASE).

• Salmonella sp 109 UB/20 CNRa: FQPS02

• *Acinetobacter baumanii* 531 UB/20 CNRa: Resistant to aztreonam

• *Klebsiella pneumoniae* 471 UB/20 CNRa: BLSE, FQPS02

• Enterobacter cloacae 543 T/20 CNRa: Wild type fluoroquinolone phenotype

Non-fermentative Enterobacteriaceae:

• *Pseudomonas aeruginosa* 469 U/20 CNRa: Hyperproduced Cephalosporinase (HCASE)

• *Pseudomonas aeruginosa* 551 UB/20 CNRa: Wild type beta-lactam phenotype

• *Pseudomonas aeruginosa* ATCC 27853: Reference strain, wild

Staphylococci:

• *Staphylococcus aureus* 211 UB/20 CNRa: Extended Spectrum Beta-lactamase (BLASE), Multi Locus Sequence Typing (MLST01)

• *Staphylococcus aureus* 483 UB/20 CNRa: BLASE, QTG phenotype (AMST 02), constitutive MLSb phenotype

• *Staphylococcus aureus* ATCC 25923: Reference strain, wild

METHODS:

Preparation of Crude Aqueous Extracts by Decoction: The crude aqueous extracts **Table 1** were prepared in an amount of 10 g of different organ powder taken up in 200 mL of distilled water. The solutions are brought to a boil for 30 min, filtered, concentrated to dryness, and stored in an oven (50 °C) for a week.

TABLE 1: CRUDE AQUEOUS EXTRACTS OBTAINED

Organ	Aqueous crude extracts code
Trunk bark	ET
Root bark	ER
Leaves	F
Equimassic mixture of the three organs	M

Preparation of Selective Extracts: The selective extracts **Table 2** were prepared by successive liquid-liquid extraction (n-hexane, chloroform, ethyl acetate, n-butanol) of each crude extract (1 g) in retaliation in 25 ml of distilled water.

TABLE 2: SELECTIVE EXTRACTS OBTAINED

	Selective extract			
	n-Hexane	Chloroform	Ethyl acetate	n-Butanol
Trunk bark	ET1	ET2	ET3	ET4
Root bark	ER1	ER2	ER3	ER4
Leaves	F1	F2	F3	F4

Phytochemical Screening:

• **Tests by Color Reactions:** The tests were carried out on crude aqueous extracts according to methodologies drawn from the literature for the identification of metabolic families: saponins, sterols, and polyterpenes [10], [11], alkaloids, coumarins, and polyphenols [12], flavonoids [13], proteins [14], tannins [15].

• **Tests by Thin Layer Chromatography (TLC):** The qualitative tests by TLC were carried out on the selective extracts (Table III) according to Kabran [16] and Kadja et al., [17]

TABLE 3: SELECTIVE AND DEVELOPING EXTRACTS USED FOR TLC

Extract	Developer
n-Hexane	Hexane/AcOEt/$CHCl_3$ (6/1/0.3; v/v/v)
Chloroform	Hexane/AcOEt/$CHCl_3$ (3/5/5; v/v/v)
Ethyl acetate	AcOH/AcOEt/$CHCl_3$ (0.5/5/5; v/v/v)
n-Butanol	n-BuOH/AcOH/EtOH (4.5/0.5/1; v/v/v)

Ethyl acetate (AcOEt), Chloroform ($CHCl_3$), Butan-1-ol (n-BuOH), acetic acide (AcOH), Ethanol (EtOH), Chromato-plate (silica gel 60. F254, Merck)

Antioxidant Test: The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical is dissolved in absolute ethanol to obtain a solution of 0.03 mg / mL. A range of concentrations (1, 0.5, 0.25, 0.125, 0.065, 0.03125 mg / mL) of the ET, ER, F, M extracts is prepared with the same solvent. In test tubes containing 1 mL of plant extract of given concentration, 2 mL of the DPPH solution are added. Tubes are incubated for 30 min in the dark, and absorbance readings are taken at 517 nm using a spectrophotometer (AL800 Spectro direct). Vitamin C of concentrations similar to those of crude aqueous extracts is the positive control [17, 18]. The percentage reduction (PR) of DPPH is calculated according to the formula

$$PR = [(A_b - A_e] \times 100$$

A_b = absorbance of blank (nm), A_e = absorbance of the sample (nm)

The median effective reduction concentrations (CR50) are determined graphically [19, 20].

Antibacterial tests:

• **Efficiency Test:** The preparation of the inoculum for the efficacy test was carried out according to Ponce [21, 22].

The commercial antibiotics Ceftriaxone (CRO), Imipenem (IPM), or Cefoxitin (FOX) were placed on the agar and served as positive controls 23.

• **Determination of the Antibacterial Parameters MBC, MIC, MBC / MIC:** The minimum bactericidal (MBC) and inhibitory (MIC) concentrations were determined using the liquid dilution method [22, 24]. The MBC / MIC ratio made it possible to specify the modality of action of the extracts against bacterial strains [25].

RESULTS AND DISCUSSION:

Extract Yields: The mass of the plant material subjected to extraction, the mass, and the yield of each aqueous crude extract are given in **Table 4**.

TABLE 4: MASSES OF ORGANS USED, MASSES AND YIELDS OF CRUDE AQUEOUS EXTRACTS

	Aqueous crude extract			
	ER	ET	F	M
Powder mass (g)	10	10	10	10
Extract mass (g)	1.66	2.36	2.84	2.17
yield (%)	16.6	23.6	28.4	21.7

ER: root bark, ET: trunk bark, F: leaves, M: equimassic mixture

Table 4 shows a variation in yield depending on the organ. A decoction of the leaves has the best yield (28.4%). This result can be compared to works in the bibliography that we have consulted. Indeed, the yield of the EF extract is higher than that obtained by Bohui 26, whose work focused on the leaves of *A. indica*, collected in Yamoussoukro (Côte d'Ivoire). These authors obtained a yield of 16.3% of the extract by decoction for 30 min of 10 g of leaves in 100 mL of distilled water. These divergent results can therefore be explained by the extraction ratio 1/10 versus 1/20. Thus, the larger this parameter, the lower the extraction yield [27]. Furthermore, we find that the yield of ER extract (16.6%) is the lowest, which seems to be explained by the fact that the water extractor would less dissolve certain phytoconstituents contained in the roots.

Phytochemical Composition of the Extracts: The results of the phytochemical screening by color reactions **Table 5** and by TLC **Table 6** to **9** of the aqueous and selective crude extracts demonstrated the phytochemical composition of *A. indica*. This preliminary step, moreover, important in the

chemical valorization of plants, makes it possible to identify families of molecules of interest.

Phytocompounds Detected by Color Reactions: Table 5 summarizes the results of the tests.

TABLE 5: PHYTOCOMPOUNDS DETECTED

	Aqueous crude extract			
Compound	**M**	**F**	**ET**	**ER**
Polyphenols	+	+	+	+
Flavonoids	+	+	+	-
Coumarins	+	+	-	+
Tannins	+	+	+	+
Saponosides	+	+	+	+
	Im=500	Im=200	Im=333	Im=500
Sterols, polyterpenes	tr	+	Tr	tr
Alkaloids	-	-	-	-
Protein	-	-	-	-

ER: root bark, ET: trunk bark, F: leaves, M: equimassic mixture; tr: in trace; (+): positive test; (-): negative test; Im: foam index

The presence of polyphenols, tannins, and saponins is brought to light in the crude aqueous extracts **Table 5**. This result is in agreement with those obtained by Bohui [26] and Biu [28], who also showed the presence of these secondary metabolites in the leaves of *A. indica* collected in Nigeria and Côte d'Ivoire. However, our results diverge from those of Ejoba [29], which showed the absence of tannins and saponins in the aqueous extract of leaves of the same species. Polyphenols are present in all the organs of the plant, and biologically, they are planted active ingredients with highly beneficial effects on health [11]. Notwithstanding their absence in the roots, flavonoids are present in the plant studied. They are antioxidants par excellence, endowed with extensive biological properties [30, 31, 32, 33, 34]. **Table 5** further indicates that tannins and saponins (at variable I'm) are present in the crude aqueous extracts. Tannins are inhibitors of bacterial growth [35]. Saponosides are hemolytic, anti-microbial, anti-bacterial [36]. Coumarins are absent in ET.

Phytocompounds Identified by TLC: To better appreciate the phytochemical composition of A. indica, we performed a TLC screening of so-called selective lighter extracts of its study organs. The results showed the existence of alkaloids, coumarins, flavonoids, polyterpenes, sterols, tannins, lupane-type triterpenes. The results are shown in **Tables 6** to **9**.

The Liebermann-Burchard reagent revealed under UV 366 nm the oleanane and ursane-type triterpenes in red, the lupane-type triterpenes in orange-yellow and the sterols in yellow and green-yellow [16]. As for Godin's reagent, it demonstrated the phytocompounds in visual light in various colorations: blue (sterols, triterpenes, and coumarins); violets (sterols and polyterpenes); yellow and orange rose (flavonoids); green (triterpenes) [17]. Normal hexane extracts showed molecular spots relating to sterols and polyterpenes and coumarins in all ER1 selective extracts; ET1 and F1 **Table 6**.

TABLE 6: PHYTOCOMPOUNDS IDENTIFIED IN THE SELECTIVE EXTRACTS WITH HEXANE

Extract	Compound	[Rf] Developer Color
ER1	Sterols, polyterpenes	[0.94] $^{g'}$ Vo
	Coumarins	[0.88] ^{c} B ; [0.71] ^{p} B ; [0.50] $^{c\,;\,p}$ B et V
	Triterpenes	[0.43] ^{g}
ET1	Sterols	[0.69] ^{e} J-V
	Coumarins	[0.84] ^{p} V ; [0.79] ^{c} J ; [0.48] ^{c}
	Sterols and polyterpenes	[0.20] ^{g} Vo
F1	Sterols and polyterpenes	[0.94] $^{g'}$ Vo ; [0.80] $^{g'}$ Vo ; [0.75] $^{gg'}$ Vo ; [0.64] $^{g'}$ Vo ; [0.39] $^{g'}$ Vo ; [0.11] $^{g'}$ Vo
	Coumarins	[0.84] ^{c} J ; [0.70] ^{p} B ; [0.46] ^{c} B

ER1: root bark; ET1: bark of the trunk; F1: leaf; a: AlCl3 (aluminum chloride) UV; a ': $AlCl_3$ visible; c: KOH; g: godin uv reagent; g ': visible Godin reagent; f: $FeCl_3$; e: Lieberman-Burchard uv reagent; e ': Lieberman-Bürchard reagent visible; P: (ACO)2Pb (basic lead acetate) UV; P ': ACO)2Pb visible; O: orange; V: green; G: gray; Vo: purple; B: blue

Neu and $AlCl_3$ reagents showed the presence of flavonoids. Indeed, they make the flavonoids appear in visual light in the form of yellow and brown molecular spots. Under UV / 366 nm irradiation, flavonoids are seen under orange, red, yellow, blue and green fluorescence 16. AlCl3 revealed these active ingredients under various fluorescences ranging from blue to brown under

UV light / 366 nm. However, in visible light, they appear yellow in color [14].

Potassium hydroxide (KOH) and basic lead acetate [(AcO)2Pb] reveal coumarins. This latter (yellow fingerprints) are seen with the naked eye after disclosure by KOH. Under UV / 366 nm, the coloration varies intensely (blue, green) [13]. (AcO)2Pb revealed them as green and blue under UV / 366 nm [16, 37].

The tannins were revealed in gray in the visible in the presence of iron (III) chloride ($FeCl_3$). Dragendorff's reagent showed the presence of alkaloids (orange or red molecular fingerprints) [17].

TABLE 7: PHYTOCOMPOUNDS IDENTIFIED IN THE SELECTIVE EXTRACTS WITH CHLOROFORM

Extract	Compound	[Rf] Developer Color
ER2	Flavonoids	[0.91] g O ; [0.65] g O ; [0.47] a J et B [0.36] a J ; [0.10] g O
	Triterpenes	[0.77] g V ; [0.57] g V ; [0.29] g V ; [0.16] g V
	Sterols	[0.16] e J
	Coumarins	[0.80] c J ; [0.49] c J ; [0.41] c'c J ; [0.34] c J
	Tannins	[0.54] f G [0.22] f G;
ET2	Flavonoids	[0.91] g O ; [0.71] g O ; [0.36] a J
	Triterpenes	[0.77] g V ; [0.57] g V ; [0.29] g V ; [0.22] g V
	Sterols and polyterpeenes	[0.65] g'g Vo ; [0.20] g Vo
	Sterols	[0.47] e J ; [0.16] e ; J
	Coumarins	[0.80] c'c J ; [0.71] g J ; [0.41] c'c J ; [0.34] c J
	Alkaloids	[0.41] d O
F2	Flavonoids	[0.91] g O ; [0.41] a' B ; [0.36] a B
	Triterpenes	[0.81] g V ; [0.29] g' V
	Coumarins	[0.80] c'c J et V ; [0.41] c J ; [0.36] c J ; [0.27] c B

ER2: root bark; ET2: bark of the trunk; F2: leaf; Rf: frontal report; a: $AlCl_3$ UV; a ': AlCl3 visible; c: KOH UV; c: KOH visible; g: UV Godin reagent; g ': visible Godin reagent; f: FeC_{l3}; e: Lieberman-Burchard UV reagent; e ': Lieberman-Burchard reagent visible; O: orange; V: green; G: gray; Vo: purple; B: blue

TABLE 8: PHYTOCOMPOUNDS IDENTIFIED IN THE SELECTIVE EXTRACTS WITH ETHYL ACETATE

Extract	Compound	[Rf] Developer Color
ER3	Sterols et polyterpenes	[0.82] g Vo ; [0.92] g Vo
	Flavonoids	[0.65] N'N J et B ; [0.57] g O ; [0.55] c V ; [0.52] N'N J et B ; [0.40] N'N J et B ; [0.31] N'N J et V ; [0.25] a V ; [0.16] N ;a V; [0.07] g ; a Ro-J et V ; [0.04] N O
	Coumarins	[0.93] p V; [0.68] p B; [0.44] c V; [0.37] cV; [0.28] pV; [0.25] c V; [0.14] c V;
	Flavonoids	[0.68] a B ; [0.65] N'N J et B ; [0.57] g O ; [0.52] N'N J et B ; [0.45] a B ; [0.40] N'N J et B ; [0.31] N'N J et V ; [0.25] a'a J et B ;
ET3		[0.17] g Ro-J; [0.16] N ;a V et B ; [0.07] g Ro-J ; [0.04] N ;g O
	Sterols and polyterpenes :	[0.92] g Vo ; [0.82] g Vo
	Coumarins	[0.14] c V
	Tannins	[0.57] fG ; [0.41] f G ; [0.18] f G ; [0.12] f G
	Sterols et polyterpenes	[0.82] g Vo ; [0.92] g Vo
	Flavonoids	[0.68] a B ; [0.65] N'N J et B ; [0.57] g O ; [0.56] a B ;
F3		[0.52] N'N J et B ; [0.40] N'N J et B ; [0.31] N'N J et V ; [0.16] N'N J et V ; [0.04] N'N J et V
	Coumarins	[0.68] pB; [0.55] c B ; [0.07] p B
	Tannins	[0.57] f G ; [0.41] f G

ER3: root bark; ET3: bark of the trunk; F3: leaf; Rf: frontal report; a: AlCl3 UV; a ': AlCl3 visible; c: KOH UV; c: KOH visible; g ': visible Godin reagent; g: UV Godin reagent; f: FeCl3; N: Neu reagent UV; N ': visible Neu reagent; O: orange; V: green; G: gray; Vo: purple; B: blue

TABLE 9: PHYTOCOMPOUNDS IDENTIFIED IN THE SELECTIVE EXTRACTS WITH N-BUTANOL

Extract	Compound	[Rf] Developer Color
ER4	Tannins	[0.53] fG
F4	Flavonoids	[0.81] a B ; [0.65] a B
ET4	Tannins	[0.85] f G; [0.25] f G

ER: root bark; ET: bark of the trunk; F: leaf; Rf: frontal report; a: $AlCl_3$ UV; f: FeCl3; O: orange; G: gray; Vo: purple

Crude Extracts Antioxidant Profile: The evaluation of the anti-free radical activity by spectrophotometry of the crude extracts was carried out using the DPPH radical, according to the vitamin C taken as a reference antioxidant **Fig. 1**. The values of CR50 **Table 9** are calculated by means of a linear regression between the concentrations and the% PR.

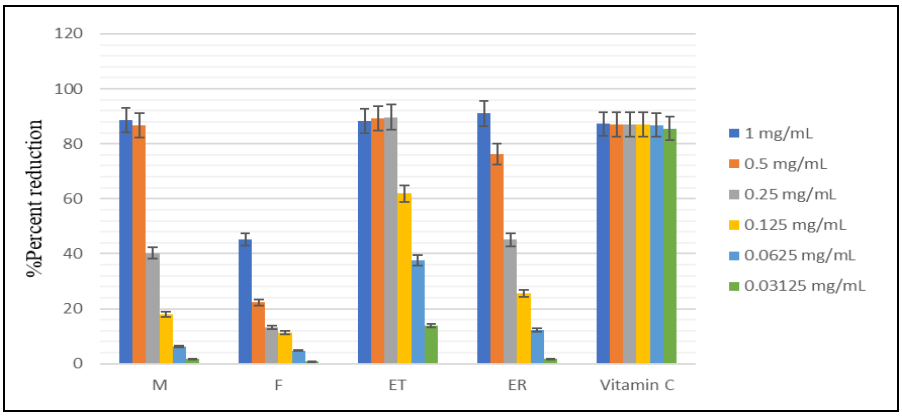

FIG. 1: ANTIOXIDANT PROFILE OF CRUDE AQUEOUS EXTRACTS ER: root bark; ET: bark of the trunk; F: leaf; M: equimassic mixture of extracts of *A. indica*

TABLE 11: CRUDE EXTRACTS ANTIOXIDANT EFFICACY

	ET	F	ER	M	Vitamin C
CR_{50} (mg/mL)	0.10	1.12	0.41	0.43	<0.03

ER: root bark; ET: bark of the trunk; F: leaf; M: equimassic mixture of extracts of *A. indica*

From this table, we see that the ET extract has a higher anti-free radical activity than the other extracts, because it has the smallest CR50 value. Indeed, the lower this value, the more the extract has a high antioxidant activity [19, 20].

Crude Extracts Bacterial Activity:

Efficiency of Crude Extracts: The different sensitivities of the extracts at 200 mg / mL with respect to the bacterial strains tested are observed. According to Ponce [21], a bacterium is said to be resistant to an extract when the diameter of its zone of inhibition around it is less than or equal to 8 mm, sensitive if this diameter is between 9 and 14 mm, very sensitive when it is between 15 and 19 mm and extremely sensitive for a diameter greater than 20 mm. The negative control used (sterile distilled water) had no effect against the bacterial strains tested. As for the positive controls, they varied depending on the bacterial strain used.

Sensitivity of Enterobacteria Strains and Antibiotic: For the class of fermentative Enterobacteria, only ET was effective against all bacterial strains **Table 12**. The M extract inhibited the strains of E. coli 470UB / 20 CNRa, E. cloacae 543T / 20 CNRa, and *A. baumanii* 531UB / 20 CNRa with diameters of zones of inhibition between 9.78 ± 0.01 and 13.14 ± 0.01 mm. On the other hand, the ER extract did not show an inhibitory effect against the bacterial strains tested because the diameters of the zones of inhibition vis-à-vis the strains are less than 8 mm. The antibiotic CRO tested gave diameters of the zones of inhibition ranging from 0 to 15.60 mm against the different bacterial strains.

TABLE 12: DIAMETERS OF THE ZONES OF BACTERIAL INHIBITION AGAINST THE CRUDE AQUEOUS EXTRACTS AND THE ANTIBIOTIC

	Aqueous crude extract				ATB
Bacterial strain	**ET**	**ER**	**F**	**M**	**CRO**
	Diameter (mm) at 200 mg / mL				
Salmonella sp 109UB/20 CNRa	15.84 ±0.07	0	0	7,29±0	16
E. coli 466TR/20 CNRa	12.32±0.02	0	8.20±0	7,34±0	0
E. coli 470 UB/20 CNRa	13.87±0.06	6.16±0	8.76±0	9.91±0.01	0
E. cloacae 543 T/20 CNRa	15.53±0	0	0	9.78±0.01	10
K. pneumoniae 471 UB/20 CNRa	11.39±0.35	0	0	0	0
A. baumanii 531 UB/20 CNRa	16.59±0.01	0	9±0	13.14±0.01	0

ET: Bark of the trunk; ER: Root bark; F: Leaf; M: equimassic mixture; CRO: Ceftriaxone; ATB: Antibiotic

Consulting the interpretation guidelines for zones of inhibition for antibiotics [38], we find that all bacterial strains have shown resistance, with the exception of the genus Enterobacter, which naturally exhibits resistance to CRO. According to this standard, for Enterobacteria, the strain is said to be resistant to CRO (30 µg) for a diameter of the inhibition zone strictly less than 22 mm and sensitive, if it is greater than or equal to 25 mm.

Sensitivity of Staphylococci to Crude Extracts and Antibiotics: The crude ET, ER, F, and M extracts showed efficacy against the bacterial strains of *S. aureus* tested **Table 13**. All strains were inhibited at 200 mg / mL with effective inhibition zone diameters between 9 ± 0 and 22 ± 0 mm. The largest diameter was observed with the crude ET extract against the bacterial strain coded for 211 UB / 20 CNRa.

The antibiotic tested, positive control, and used in treating pathologies linked to staphylococci, gives zone diameters of inhibition ranging from 20 to 26 mm. Consulting the interpretation guidelines for zones of inhibition for antibiotics, we find that the bacterial strains have shown resistance, with the exception of the ATCC reference. According to CASFM38, a strain of Staphylococcus is said to be sensitive to FOX (30 µg), if the diameter of the zone of inhibition of the antibiotic is greater than or equal to 22 mm and resistant in the opposite case.

TABLE 13: DIAMETERS OF THE ZONES OF BACTERIAL INHIBITION VIS-À-VIS THE CRUDE AQUEOUS EXTRACTS AND THE ANTIBIOTIC

	Aqueous crude extract				ATB
Bacterial strain	**ET**	**ER**	**F**	**M**	**FOX**
	Diameter (mm) at 200 mg / mL				
S.aureus 211 UB/20 CNRa	22±0	14.03±0.06	10.06±0.06	16±0.1	20
S.aureus 483 UB/20 CNRa	21.1±0.1	13±0	9±0	15±0	20
S.aureus ATCC 25923	17.03±0.15	17.07±0.06	15±0	13.03±0.06	26

ET: Bark of the trunk; ER: Root bark; F: Leaf; M: equimassic mixture; FOX: Cefoxitin, ATB: Antibiotic

Sensitivity of Non-fermentative Enterobacteria to Crude Extracts and Antibiotic: The extracts and the antibiotic is active against bacterial strains of *P. aeruginosa* with the exception of *P. aeruginosa* 469 UB / 20 CNRa. For all the extracts tested as effective, the diameters of the zones of inhibition are between 14.65 ± 0.01 and 28 ± 1 mm **Table 14**. As for the reference antibiotic tested for IPM, it has diameters of the zones of bacterial inhibition ranging from 26 to 33 mm.

TABLE 14: DIAMETERS OF THE ZONES OF BACTERIAL INHIBITION VIS-À-VIS THE CRUDE AQUEOUS EXTRACTS AND THE ANTIBIOTIC

	Aqueous crude extract				ATB
Bacterial strain	**ET**	**ER**	**F**	**M**	**IPM**
	Diameter (mm) at 200 mg / mL				
P.aeruginosa 469UB/20 CNRa	6±0	6±0	6±0	6±0	29
P.aeruginosa 551UB/20 CNRa	14.65±0,01	23±0	23.03±0,06	28±1	33
P.aeruginosa ATCC 27853	22±0	17±0,1	21±0	21±0	52

ET: Bark of the trunk, ER: Root bark, F: Leaf, M: equimassic mixture, IPM: Imipenene, ATB: Antibiotic

The diameters of the bacterial inhibition zone of antibiotics confirm the different phenotypes of the bacterial strains obtained previously. MIC and CMB were determined for extracts exhibiting antibacterial activity.

Effect of Effective Extracts by Diffusion in Liquid Medium: After 24 h of incubation at 37 ° C, a progressive decrease in bacterial growth in the wells of the microplates as a function of the different concentrations of the extracts tested compared to the growth control Tc is observed.

The MIC and MBC values determined with regard to the bacterial strains are grouped together in Tables XV-XVII. The MBC / MIC report made it possible to specify the modality of action of the extracts. If this ratio is less than or equal to 2, the substance is said to be bactericidal and strictly greater than 2; the substance concerned is considered bacteriostatic [25]. Among the three study organs, ET extract appears to be the most effective against Enterobacteria. On the other hand, to achieve the bactericidal effect against the *E. coli*

470 UB / 20 CNRa strains, an equimassic mixture of these organs is necessary. Against staphylococci and non-fermentative Enterobacteria, all decocts would be bactericidal with the exception of F against S. aureus 211 UB / 20 CNRa, S. aureus 483 UB / 20 CNRa, and P. aeruginosa 551 UB / 20 CNRa.

TABLE 15: ANTIBACTERIAL PARAMETERS OF CRUDE AQUEOUS EXTRACTS AGAINST ENTEROBACTERIA

Bacterial strain	Aqueous crude extract	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	Interpretation
Salmonella sp 109 UB/ CNRa	ET	12.5	ND	ND	Bactriostatic
E. coli 466 TR/20 CNRa	ET	12.5	25	2	Bactericidal
E. coli 470 UB/20 CNRa	ET	12.5	ND	ND	Bactriostatic
	M	50	50	1	Bactericidal
E. cloacae 543 T/20 CNRa	ET	12.5	12,5	1	Bactericidal
	M	50	ND	ND	Bactriostatic
K.pneumoniae 471 UB/20 CNRa	ET	25	50	2	Bactericidal
A.baumanii 531 UB/20 CNRa	ET	6.25	12,5	2	Bactericidal
	F	50	ND	ND	Bactriostatic
	M	50	100	2	Bactericidal

ET: Bark of the trunk, F: Leaf; M: equimassic mixture, ND: Not determined

TABLE 16: ANTIBACTERIAL PARAMETERS OF CRUDE AQUEOUS EXTRACTS AGAINST STAPHYLOCOCCI

Bacterial strain	Aqueous crude extract	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	Interpretation
	ET	3.125	3.125	1	Bactericidal
S.aureus 211 UB/20 CNRa	ER	3.125	6.25	2	Bactericidal
	F	100	ND	ND	Bactriostatic
	M	3.125	6.25	2	Bactericidal
	ET	3.125	3.125	1	Bactericidal
S.aureus 483 UB/20 CNRa	ER	3.125	6.25	2	Bactericidal
	F	100	ND	ND	Bactriostatic
	M	100	ND	ND	Bactriostatic
	ET	3.125	3.125	1	Bactericidal
S.aureus ATCC	ER	6.25	12.5	2	Bactericidal
	F	25	50	2	Bactericidal
	M	12.5	12.5	1	Bactericidal

ET: Bark of the trunk, F: Leaf, M: equimassic mixture, ND: Not determined

TABLE 17: ANTIBACTERIAL PARAMETERS OF CRUDE AQUEOUS EXTRACTS AGAINST NON-FERMENTATIVE ENTEROBACTERIA

Bacterial strain	Aqueous crude extract	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	Interpretation
	ET	12.5	25	2	Bactericidal
P.aeruginosa 551 UB/20 CNRa	ER	50	100	2	Bactericidal
	F	100	ND	ND	Bactriostatic
	M	25	50	2	Bactericidal
	ET	6.25	12.5	2	Bactericidal
P.aeruginosa ATCC 27853	ER	25	50	2	Bactericidal
	F	25	50	2	Bactericidal
	M	50	50	1	Bactericidal

ET: Bark of the trunk, F: Leaf, M: equimassic mixture, ND: Not determined

The determination of the antibacterial parameters made it possible to characterize the nature of the effect revealed by the extracts on a given microorganism. The bactericidal action observed could be explained by the presence of secondary metabolites contained in the extracts tested. These secondary phytocompounds are said to be endowed with antibacterial properties, which would attest to the use of the plant in traditional medical practice.

CONCLUSION: The phytochemical screening of *Azadirachta indica*, based on specific qualitative tests, made it possible to identify metabolic families (alkaloids, coumarins, flavonoids,

polyterpenes, saponosides, sterols, tannins), which are endowed with proven therapeutic properties. The antioxidant activity of the crude extracts evaluated with respect to the DPPH radical, showed that the aqueous decoction of the bark of the trunk (ET) has the most significant antioxidant efficacy (CR50 = 0.10 mg / mL), and this, in comparison to vitamin C.

Biological tests of organ extracts of the said plant against various bacterial strains have demonstrated its sensitivity, its antimicrobial profile, and this, depending on the types of extract, plant organ and bacterial strain. The extracts generate areas of antibacterial activity with diameters varying between 9 and 28 mm at 200 mg / mL. The results obtained at the end of the present study are indisputable proofs that demonstrate the validity of the popular and traditional medicine use of *Azadirachta indica*.

ACKNOWLEDGEMENT: The authors are thankful Pastor Institute of Côte d'Ivoire.

CONFLICTS OF INTEREST: All authors declare that there are no conflicts of interest.

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How to cite this article:
Brice KA, Atsain-Allangba MR, Stéphane KK, Mamyrbékova-Békro JA and Yves-Alain B: Phytochemical screening, antioxidant and antibacterial activities of extracts of *Azadirachta indica* a. juss (meliaceae) (côte d'ivoire). Int J Pharm Sci & Res 2022; 13(1): 417-26. doi: 10.13040/IJPSR.0975-8232.13(1).417-26.