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ANTIBACTERIAL ACTIVITY OF AQUEOUS AND METHANOL EXTRACTS OF *Jatropha tanjorensis* AND A*dansonia digitata* AGAINST SELECTED CLINICAL BACTERIA PATHOGENS

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ABSTRACT

The powdered plant parts (bark, stem, and leaf) were extracted with methanol, and cold and hot water using the maceration method. A modified agar well diffusion technique was used for the assessment of the antibacterial activities of the aqueous and methanol extracts of J. tanjorensis and A. digitata. The methanol extract of A. digitata bark gave the highest yield of 75 % while the lowest yield was observed with the cold water extract of A. digitata bark of 33 %. The phytochemical screening showed an abundance of alkaloids, terpernoids, saponins, flavonoids, tannin, glycoside, and phenol. The bark, leaf, and stem of J. tanjorensis and A. digitata showed varying degrees of antibacterial activities. against the Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa, Proteus spp, and Klebsiella spp) and Gram-positive bacteria (Staphylococcus aureus, Streptococcus pyogenes and Streptococcus pneumonia). The bark, stem, and leaf extract of J. tanjorenis significantly ($p < 0.05$) inhibited all the pathogens except P. aeruginosa and S. aureus.The result of the minimum bactericidal concentration MBC for the combined extracts of A. digitata and J. tanjorenis subfractions showed that the extracts have greater antibacterial activities at concentrations not lesser than 100 mg/ml. Thus, J. tanjorensis and A. digitata could be used as potent herbal remedies to mitigate the adverse effects of Gram-negative and positive clinical pathogens. Thus, the aim of this study was to evaluate the antibacterial activity of aqueous and methanol extracts of Jatropha tanjorensis and Adansonia digitata against selected Clinical bacteria pathogens.

Keywords*:* Antibacterial, maceration, *Jatropha tanjorensis,* A*dansonia digitata***,*** phytochemical, clinical pathogens

INTRODUCTION

The global increase in multi-drug resistance by bacterial pathogens to conventional antibiotics has become a major public health concern and has posed a lot of challenges in the treatment of infectious diseases recently. This has prompted scientists to search for newer strategies to curb this menace. One such way is the use of medicinal herbs with

antimicrobial potential and less toxic than conventional antibiotics.

Adansonia digitata L., also known as African baobab, belongs to the order Malvales and the family Bombacaceae (USDA, 2019). African baobab is one of the eight species of *Adansonia* and the only one native to Africa (Heuzé*et al.,* 2016). It is the most widespread tree species of the *Adansonia* genus. The name baobab is probably derived from the Arabic words 'buhibab' meaning 'fruits with many seeds'. Its scientific name *A. digitata*was given by Carl von Linné in honour of the French scientist Michel Adanson who was the first European botanist to see and describe the tree in its native habitat. The term '*digitata*' refers to the shape of the tree leaves (Gebauer*et al.,* 2016). In Nigeria, the Igbos call it 'oyili-akpu', the Hausas call it 'kuka', the Yorubas call it 'ose' (Iwu, 2014), while the Binis call it 'usi' or 'ushi'. The Okoo people of Kwara State, Nigeria call the leaves 'luru' (Amusa, 2017).

Samatha*et al.,* (2017), observed that extracts from different parts of *A. digitata* can be used as potential sources of antibacterial agents to combat infectious diseases. This was collaborated by other studies where *A. digitata and J. Tanjorensis* exhibited broad spectra of activities against Gram-positive and negative bacteria (Kumar *et al.,* 2016, Samatha*et al.*, 2017, Abdallah and Ali 2019). Similar antibacterial activities have been reported of the crude extracts of *J. tanjorensis* (Viswanathan*et al*., 2012, Babayemi*et al.*, 2021).*J. tanjorensis* is a perennial herb that belongs to the family *Euphorbiaceae.* Locally, it is called "*Hospital too far*" in Pidgin English; or Catholic vegetable, and "Iyana-ipaja" (Yoruba) (Babayemi*et al*., 2021). The phytochemical constituents of *A. digitata* and *J. tanjorensis* plant could be the reason behind its antibacterial activities. Phytochemical screening performed on the various parts of *A. digitata* and *J. tanjorensis* extracts indicated that they are rich in glycosides, flavonoids, saponins, alkaloid, steroids, terpenoid, tannins, volatile matter, reducing sugars, phlobatannins and anthraquinones (Datsugwai and Yusuf 2017, Daniyan*et al.*, 2018, Elinge*et al.*, 2020).

It is already well known that either of *J. tanjorensis* or *A. digitata* possesses antibacterial activity. A lot of people especially those in the developing world now depend on traditional medicines like *J. tanjorensis* and *A. digitata* to cure these resistant bacteria (Oyebode et al., 2016). However, many are not aware of what gives these plants their desirable antibacterial activities and also do not know if mixing the both plants will give a more desirable and synergistic antibacterial effect. This study seeks to address the combined effects of these plants on bacterial isolates especially antibiotic resistant strains. This is because the issues these strains cause to the world health are becoming overwhelming. This project addresses the problem of lack of information on the combined effects of these plants extracts on bacterial infections.

MATERIALS AND METHODS

Collection and Identification of Plants

The bark, stem, and leaf of *A. digitata* and *J. tanjorensis* were obtained from farmland in Enugu state from April 2021 to July 2021. They were authenticated by Mr. Felix Nwafor, a taxonomist in the Department of Pharmacognosy, University of Nigeria, Nsukka (UNN), Enugu, Enugu State. The voucher specimens of A*dansonia digitata* L and *Jatropha tanjorensis* were deposited at the departmental herbarium with the following respective voucher numbers UNN/04/0523A and UNN/04/0522C.

Preparation of Plant Samples

The bark, stem, and leaf of *A. digitata* and *J. tanjorensis* were rinsed thoroughly in running tap water. The bark and stem were sun-dried while the leaves were dried at room temperature. The different plant parts were ground into fine powder with a mechanical grinder. The powdered plant parts were then stored in airtight polythene bags.

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Aqueous and methanol extraction of the active ingredient

This was done according to the method of Udoh*et al*., (2012) with little modifications. The bark, stem, and leaf powder of A. *digitata* and *J. tanjorensis* were extracted with methanol, cold, and hot water. 100 g portion of each of the powdered plant parts was macerated in a total of 0.5 L of 95 % methanol for 72 h with intermittent agitations until exhaustively extracted at room temperature. The methanol extracts were filtered and the filtrates were concentrated under reduced pressure using a rotator evaporator to allow for evaporation to dryness. The dried crude extracts were stored in air-tight containers. For the cold water extraction, 100 g of each of the powdered plant parts were soaked in 0.5 L of distilled water and left at room temperature (25 ^{o}C – 28 ^{o}C) for 24 h with occasional agitation. Similarly, for the hot aqueous extraction, 100 g of each of the powdered plant material was soaked in 0.5 L of boiling water and was kept at 100 ^{o}C for 1 h. The extract was filtered and the filtrates were concentrated by evaporation in a steady air current. The yields were calculated by the following formula:

$$\% \text{ Yield (Recovery of extract)} = \frac{Final\ weight\ of\ extract\ recovered\ after\ extraction\ (g)}{Initial\ weight\ of\ plant\ powder\ (g)} \times 100$$

Sterility test of the extracts

A modified method by Babayemi*et al*., 2021 was adopted. Instead of absolute ethanol, the extracts were dissolved in 10 %v/v DMSO. To test for the presence of microbial growth and contaminants, a 10-fold dilution of each extract was made using sterile deionized distilled water and filtered sterilized through a 0.45 µm millipore membrane filter (Millipore, India). Briefly, the extract was mixed thoroughly using a sterile spatula and 1 g was weighed into 10 ml of sterile DMSO. The dissolved extract was filtered and sterilized through a 0.45 µm millipore membrane filter (Millipore, India). Subsequently, 1 ml of the extract was re-dissolved in 9 ml of sterile deionized distilled water and serially diluted to 10^{-6} dilutions. Aliquot (100 µL) of the 10^{-3} to 10^{-6} dilutions of each extract were transferred aseptically into already prepared Petri dishes containing nutrient agar and spread uniformly over the plate using a sterile glass spreader. The inoculated plates were incubated at 37°C for 24 h. The absence of microbial growth in the extracts after incubation indicated that the extracts were sterile.

Phytochemical Screening

A preliminary phytochemical analysis of the plant parts was performed and involved testing for the presence or the absence of secondary metabolites like alkaloids, flavonoids, terpenoids, tannins, saponins, phenols, and glycosides using standard procedures of Harbourne (1998) and Evans (1998). Qualitative analysis was conducted to identify the medicinal potentials of the two plants. Twenty-five grams (25 g) of each sample was soaked in 100 ml of different solvents such as methanol, distilled water, n-hexane, ethyl acetate, and ethanol for 24 h. The extract was decanted and heated for 3 mins to concentrate it. The presence or absence of secondary metabolites like alkaloids, flavonoids, terpenoids, tannins, saponins, phenols, and glycosides was checked using standard methods.

Test Microorganisms

Clinical bacterial isolates were obtained from the Medical Microbiology laboratory of the University of Nigeria Teaching Hospital (UNTH), Enugu, Enugu State, Nigeria. They were inoculated onto nutrient agar slants, blood agar slants, and chocolate agar slants. The bacterial isolates used were *Pseudomonas aeruginosa, Staphylococcus aureus, Proteus* spp, *Klebsiella*spp, *Streptococcus pyogenes, Escherichia coli,* and *Streptococcus pneumoniae*. The bacterial isolates were confirmed by standard bacteriological methods and purified by three

successive sub-cultures in nutrient and blood agar. Purified cultures were stored on a nutrient agar slant at 4°C. Before use the organisms were activated by successive daily sub-culturing onto fresh agar slant for 72 h. Overnight (18-24 h) cultures in nutrient broth were standardized with 0.5 McFarland standards (Chessbrough, 2006).

Standard inoculum preparation

A 100 µL of overnight grown cultures of each pathogen were inoculated into 10 mL of Brain Heart Infusion broth (pH 6.4) and incubated aerobically at 37 °C for 24 h. The supernatant was prepared by centrifugation (10,000 g for 2 min) and then filtered through a 0.45 µm filter (Millipore, India). The filtrates were standardized to 0.5 MarFarland turbidity standard (10^6 cfu/mL) and adjusted accordingly with a spectrophotometer (optical density, OD 600 nm). The filtrates were used immediately.

Assay of susceptibility of microorganisms to Extracts

The modified agar well diffusion technique of Balouiri*et al*.(2016), was used for the assessment of antibacterial activities of methanolic and aqueous bark, stem, and leaf extract of *J. tanjorensis* and *A. digitata*. A solution of the methanol extracts (100 mg/ml) was prepared in Dimethyl sulfoxide (DMSO) and a solution of (100mg/ml) aqueous extracts was made in distilled water. Molten Mueller Hinton agar (19.9 ml) was seeded with 0.1 ml of the standardized broth culture of bacteria and was allowed to set. After solidification, an 8 mm sterile cork borer was used to make a total of five wells on the agar. Two drops of 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml concentrations of each of the extracts were carefully added into the wells. Two drops of a 2-fold dilution of DMSO were added in the middle well as negative control. All the Petri dishes were left at room temperature for 1 h for diffusion of the extract before incubating at 37°C for 24 h. The determination of antibacterial activity was based on the measurement of the inhibition zone diameter (IZD) formed using a meter rule. The experiment was performed in triplicates and the mean reading was taken as the IZD. The extracts that yielded the highest IZD from *A. digitata* and *J. tanjorensis* were used for combination studies. The plant extracts of *A. digitata* and *J. tanjorensis* with the highest zones of inhibition were mixed homogenously and dilutions were made in the same order; 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml.

Combined minimum inhibitory concentration (MIC) and determination of minimum bactericidal concentration (MBC)

The MIC of the combined extracts of the best of both plant extracts was tested on the test organisms using the modified broth dilution method. Both extracts were uniformly mixed in a 1:1 ratio to give a final stock concentration of 200 mg/ml. A 2-fold serial dilution was used to dilute the combined plant extracts (stock) to make dilutions of: 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml. Two millitres (2 ml) of the stock were mixed with 2 ml of nutrient broth thereby diluting it to 100 mg/ml. Into each of these concentrations, 200 µL of the test organisms were introduced accordingly. A negative control was prepared using the nutrient broth and the plant extract only. These were incubated at 37 °C for 24 h and observed for the least concentration which inhibited the organism growth. The lowest concentration that showed no growth was considered to be the MIC (Habtom and Gebrehiwot, 2019).

The test tubes indicating the MIC and other preceding tubes (also showing inhibition of the bioactive compound) were streaked on Mueller Hinton Agar (MHA) plates and incubated for about 18 h at 37 ^{o}C. The absence of growth after the incubation, was indicative of MBC of the combined extracts.

Statistical Analysis

The result obtained was analyzed by one-way Analysis of variance (ANOVA) followed by Dunnet's multiple comparisons Posthoc test

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using Graph Pad Prism version 7 software. The values are expressed as mean ± standard error of the mean (SEM). Statistical significance was accepted at $P \leq 0.05$.

RESULT

Extraction yields of *A. digitata* and *J. tanjorensis*

The percentage yield of each extract of *A. digitata* and *J. tanjorensis* is shown inTable 1. The percentage yield of *A. digitata* ranged from 16 % in cold water extract of *A. digitata* leaf (CLA) to 75 % in methanol extract of *A. digitata* bark (MBA) subfractions. Similarly, among the sub-fractions of *J. tanjorensis, the* methanol extract of *J. tanjorensis* leaf (MLJ) gave the highest yield of 70 % while cold water extract of *J. tanjorensis* stem had the lowest yield of 24 %.

Table 1. Percentage Yield of the crude extracts

Plant extract	Mass of dried plant powder (g)	The final mass of powdered extracts (g)	Percentage yield (%)
MBA	100	75.08	75
MLA	100	55.05	55
MSA	100	70.03	70
HBA	100	65.05	65
HLA	100	60.04	60
HAS	100	55.54	56
CBA	100	33.09	33
CLA	100	15.60	16
CSA	100	32.45	32
MBJ	100	65.42	65
MLJ	100	70.17	70
MSJ	100	45.06	45
HBJ	100	65.68	66
HLJ	100	45.56	46
HSJ	100	45.05	45
CBJ	100	35.01	35
CLJ	100	25.07	25
CSJ	100	24.40	24

Note: **MBA**= Methanol extract of *A. digitata* bark; **MLA**= Methanol extract of *A. digitata* leaf; **MSA**= Methanol extract of *A. digitata* stem; **HBA**= Hot water extract of *A. digitata* bark; **HLA**= Hot water extract of *A. digitata* leaf; **HAS** = Hot water extract of *A. digitata* stem; **CBA**= Cold water extract of *A. digitata* bark; **CLA**= Cold water extract of *A. digitata* leaf; **CSA** = Cold water extract of *A. digitata* stem; **MBJ** = Methanol extract of *J. tanjorensis* bark; **MLJ** = Methanol extract of *J. tanjorensis* leaf; **MSJ** = Methanol extract of *J. tanjorensis* stem; **HBJ** = Hot water extract of *J. tanjorensis* bark; **HLJ**= Hot water extract of *J. tanjorensis* leaf; **HSJ** = Hot water extract of *J. tanjorensis* stem; **CBJ**= Cold water extract of *J. tanjorensis* bark; **CLJ**= Cold water extract of *J. tanjorensis* leaf; **CSJ**= Cold water extract of *J. tanjorensis* stem.

Qualitative phytochemical constituents of *A. digitata* and *J. tanjorensis* extracts

The qualitative phytochemical studies on the various extracts of *A. digitaae* and *J. tanjorensis* are shown in Table 2. The preliminary phytochemical test of both extracts revealed the presence of secondary metabolites such as alkaloids, saponins, tannins, glycosides, flavonoids, phenols, and terpenoids. Saponin was mosertely present in all the subfractions. Phenol was abundantly present in *A. digitata* leaf methanol extract (ALM), *A. digitata* stem methanol extract (ASM), *A. digitata* bark aqueous extract (ABAq) and *A. digitata* bark methanol extract (ABM). Interestingly, *J. tanjorensis* bark methanol extract (JBM) have a greater preponderance of all the phytochemical constituents than other subfractions.

Table 2. Qualitative Phytochemical Analysis

Extract	Saponin	Tannin	Flavonoid	Glycoside	Terpenoid	Phenol	Alkaloid
JLAq	+	++	++	-	+++	++	+++
JLM	++	+++	++	+	+++	+	+
JSAq	++	++	+++	-	++	-	+++
JSM	++	+++	+++	++	+++	-	++
JBAq	+	++	++	+	+	-	+++
JBM	++	+++	+++	++	+++	++	++
ALAq	++	+	+++	-	++	++	+++
ALM	++	++	+	++	+++	+++	+
ASAq	+	++	++	+	+	++	+++
ASM	++	+++	+	++	+++	+++	++
ABAq	+	++	++	++	++	+++	+++
ABM	++	+++	-	++	+++	+++	++

Note: - absent, + present, ++ moderately present, +++ abundantly present; JLAq = *J. tanjorensis* leaf aqueous extract, JLM = *J. tanjorensis* leaf methanol extract, JSAq = *J. tanjorensis* stem aqueous extract, JLM = *J. tanjorensis* stem methanol extract, JLAq = *J. tanjorensis* bark aqueous extract, JBM = *J. tanjorensis* bark methanol extract; ALAq = *A. digitata* leaf aqueous extract, ALM = *A. digitata* leaf methanol extract, ASAq = *A. digitata* stem aqueous extract, ASM = *A. digitata* stem methanol extract, ABAq = *A. digitata*bark aqueous extract, ABM = *A. digitata* bark methanol extract.

Mean inhibition zone diameter (IZD) of aqueous extracts of *A. digitata* and*J. tanjorensis* against Gram-positive and Gram negative bacteria

Table 3 A – D shows the mean IZD of aqueous extracts of *A. digitata* and *J. tanjorensis* against Gram-positive and Gram negative bacteria. The cold water extract of *A.digitata* against Gram-positive and Gram negative bacteria is shown in Table 3 A. The cold water extract *of A. digitata* showed the weakest inhibition compared to other extracts against all the pathogens at the various concentrations tested.

Table 3 A. Mean inhibition zone diameter of cold water extract of *A. digitata* against Gram-positive and Gram negative bacteria

Crude extract	**Conc. (mg/ml)**	**Inhibition Zone Diameter, IZD (mm)**						
		P. aeruginosa	***S. aureus***	***Proteus sp***	***Klebsiellas p***	***S. pyogenes***	***S. pneumoniae***	***E. coli***
Bark	100	12 ±1.0^{ab}	10±1.0^{a}	8±1.0^{a}	8±0.5^{a}	12±0.5^{ac}	10±0.0^{b}	13±0.5^{ab}
	50	12±1.0^{a}	10±1.0^{c}	8±0.0^{a}	8±0.0^{a}	10±0.0^{a}	8±0.5^{b}	12±0.5^{b}
	25	10±1.0^{a}	8±1.0^{b}	8±0.0^{a}	8±0.5^{a}	8±0.5^{a}	8±1.0^{a}	10±0.0^{b}
	12.5	8±1.0^{a}	8±1.0^{a}	8±0.5^{a}	8±0.5^{a}	8±0.0^{a}	8±0.0^{a}	10±1.0^{a}
Leaf	100	12±1.0^{ab}	8±1.0^{a}	8±0.0^{a}	10±0.0^{a}	8±0.5^{a}	10±0.5^{b}	12±0.5^{a}
	50	10±1.0^{b}	8±1.0^{a}	8±0.0^{a}	10±0.0^{a}	8±0.0^{a}	8±0.5^{b}	9±0.0^{a}
	25	8±1.0^{ab}	8±1.0^{a}	8±0.0^{a}	8±0.5^{a}	8±0.0^{a}	8±1.0^{b}	8±1.0^{a}
	12.5	8±1.0^{a}	8±1.0^{a}	8±0.5^{a}	8±0.0^{a}	12±0.5^{c}	8±0.0^{a}	8±0.0^{a}
Stem	100	12±0.5^{a}	8±0.0^{a}	8±1.0^{a}	8±0.5^{a}	12±0.0^{b}	8±0.5^{a}	8±1.0^{c}
	50	12±0.5^{a}	8±0.0^{a}	8±0.5	8±0.0^{a}	10±0.0^{a}	8±0.5^{a}	8±0.0^{a}
	25	10±0.0^{a}	8±0.5^{a}	8±0.0^{a}	8±0.5^{a}	9±0.5^{b}	8±0.0^{a}	8±0.5^{a}
	12.5	8±0.0^{a}	8.0±0.0^{a}	8±0.5^{a}	8±0.0^{a}	9±0.0$^{a\,c}$	8±0.5^{a}	8±0.0^{a}
Positive control	CIP (30µg/l)	32±0.5^{ab}	30±0.5^{ac}	35±0.5^{ab}	35±0.5^{ab}	30±0.5^{ab}	33±0.5^{ab}	32±0.5^{ab}
Negative control	DMSO (10 %v/v)	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}

Note: Values represent the means (to the nearest whole number) ± standard error of mean (SEM) of triplicate observations. conc. = concentration; CIP = Ciprofloxacin; DMSO = Dimethyl sulfoxide, Superscripts of the same letter in a column are not significantly different at $p \leq 0.05$. n = 3.

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The mean IZD ofhot water extract of *A.digitata* against Gram-positive and Gram-negative bacteria is shown inTable 3 B. At concentrations of 100 mg/ml, the hot water extract of *A. digitata* leaf, bark, and stem had higher antibacterial activity against *S. aureus, Proteus* spp, *Klebsiella*spp with the highest IZD of 40 mm. The antibacterial susceptibility of the hot water extract of *A. digitata* stem revealed strong activity of the stem and leaf subtractions against *P. aeruginosa with IZD range of* 40 mm and 36 mm, at concentrations of 100 and 12.5 mg/ml. Similarly, the bark and stem fractions of the hot water extract of *A. digitata* exhibited strong antibacterial with IZD of 30 and 36 mm respectively of 100 mg/ml.

Table 3 B. Mean inhibition zone diameter of hot water extract of *A. digitata* against Gram-positive and Gram negative bacteria

Crude extract	Conc (mg/ml)	Inhibition Zone Diameter, IZD (mm)						
		P. aeruginosa	***S. aureus***	***Proteus sp***	***Klebsiellasp***	***S. pyogenes***	***S. pneumonia***	***E. coli***
Bark	100	21±1.0^{b}	8±0.0 ^{a}	40±0.5^{ab}	8±1.0 ^{a}	38±0.5^{ab}	8±0.0 ^{a}	30±1.0^{ab}
	50	18±0.5 ^{a}	8±0.5 ^{a}	37±1.0 ^{b}	8±0.5 ^{a}	25±1.0 ^{b}	8±1.0 ^{a}	30±0.5 ^{b}
	25	12±0.5 ^{a}	8±1.0 ^{a}	34±0.5^{ab}	8±1.0 ^{a}	20±0.5 ^{a}	8±0.0 ^{a}	28±1.0 ^{a}
	12.5	8±1.0 ^{a}	8±0.5 ^{a}	29±1.0 ^{a}	8±0.5 ^{a}	8±1.0 ^{a}	8±0.5 ^{a}	25±0.5 ^{a}
Leaf	100	36±0.0 ^{a}	40±1.0 ^{a}	37±0.0^{ab}	26±0.5^{ab}	39±0.5 ^{ac}	8±1.0 ^{a}	36±1.0 ^{ac}
	50	35±1.0^{ab}	38±0.5^{ab}	38±1.0 ^{b}	20±1.0^{ab}	35±1.0^{ab}	8±0.0 ^{a}	35±0.5^{ab}
	25	35±1.0^{ab}	32±0.5 ^{a}	38±0.0^{ab}	8±0.5 ^{a}	30±0.5 ^{a}	8±1.0 ^{a}	32±0.0 ^{a}
	12.5	25±0.5 ^{a}	32±1.0 ^{a}	37±0.0 ^{a}	8±1.0 ^{a}	27±0.5^{ab}	8±0.0 ^{a}	22±1.0 ^{a}
Stem	100	40±1.0^{bc}	8±0.0 ^{a}	8±1.0 ^{a}	40±0.0^{ab}	8±1.0 ^{a}	30±0.0^{ab}	35±0.5 ^{a}
	50	39±0.5^{ab}	8±1.0 ^{a}	8±1.0 ^{a}	30±0.0 ^{ac}	8±1.0 ^{a}	8±1.0 ^{a}	34±0.0 ^{a}
	25	31±0.5 ^{b}	8±0.5 ^{a}	8±0.0 ^{a}	28±1.0 ^{a}	8±0.0 ^{a}	8±0.5 ^{a}	30±1.0^{ab}
	12.5	28±1.0 ^{a}	8±1.0 ^{a}	8±1.0 ^{a}	20±0.0 ^{ac}	8±1.0 ^{a}	8±1.0 ^{a}	28±0.5 ^{a}
Positive control	CIP (30µg/l)	40±0.5^{ab}	33±0.5 ^{ac}	42±0.5^{ab}	45±0.5 ^{ac}	38±0.5^{ab}	31±0.5 ^{ac}	37±0.5^{ab}
Negative control	DMSO (10 %v/v)	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}

Note: Values represent the means (to the nearest whole number) ± standard error of mean (SEM) of triplicate observations. conc. = concentration; CIP = Ciprofloxacin; DMSO = Dimethyl sulfoxide, Superscripts of the same letter in a row are not significantly different at $p \leq 0.05$. n = 3

The Mean IZD of cold water extract of *J. tanjorensis* against Gram-positive and Gram negative bacteria is shown in Table 3 C. The cold water extract of extract of *J. tanjorens* showed a poor antibacterial activity against the pathogens. However, the highest IZD of 15 mm and 14 mm was observed at 100 mg/ml the the cold water extract of *J. tanjorensis* bark against *P. aeruginosa and S. pyogenes.*

Table 3 C: Mean inhibition zone diameter of cold water of extract of *J. tanjorenis* against Gram-positive and Gram negative bacteria

Crude extract	Conc. (mg/ml)	Inhibition Zone Diameter, IZD (mm)*						
		P. aeruginosa	***S. aureus***	***Proteus sp***	***Klebsiellasp***	***S. pyogenes***	***S. pneumoniae***	***E. coli***
Bark	100	15±0.0^{ab}	9±0.5 ^{a}	9±1.0 ^{a}	8±0.0 ^{a}	14±0.5^{ab}	10±1.0^{ab}	12±1.0 ^{ac}
00	50	10±1.0 ^{a}	8±1.0^{ab}	9±0.0 ^{a}	8±0.0 ^{a}	11±0.0 ^{b}	9±0.0 ^{a}	10±1.0 ^{b}
	25	6±0.0 ^{b}	8±0.0 ^{a}	8±0.0 ^{a}	8±0.0 ^{a}	9±1.0 ^{b}	9±1.0 ^{a}	7±0.0^{ab}
	12.5	5±1.0^{a}	8±1.0^{ab}	8±1.0 ^{a}	8±1.0 ^{a}	5±0.5 ^{a}	8±0.5 ^{a}	5±0.5 ^{a}
Leaf	100	12±0.0 ^{ac}	9±0.5 ^{a}	15±0.0 ^{a}	9±0.5 ^{ac}	8±0.0 ^{a}	12±0.0 ^{a}	10±1.0^{ab}
	50	10±0.5 ^{b}	9±0.0 ^{a}	14±0.5 ^{b}	9±0.0^{ab}	8±1.0 ^{a}	11±0.5 ^{a}	8±0.0 ^{a}
	25	7±1.0^{ab}	9±0.5 ^{a}	12±0.0 ^{b}	6±1.0^{ab}	8±1.0 ^{a}	10±0.0 ^{a}	8±0.5 ^{a}
	12.5	6±0.0 ^{a}	9±0.5 ^{a}	12±0.5 ^{a}	8±0.5 ^{a}	8±0.5 ^{a}	12±0.5 ^{a}	8±0.0 ^{a}
Stem	100	12±1.0^{ab}	11±1.0 ^{a}	8±0.0 ^{a}	10±0.0^{ab}	9±0.0 ^{a}	10±0.0 ^{a}	8±0.0 ^{a}
	50	10±0.0 ^{a}	9±0.5 ^{ac}	8±0.5 ^{a}	10±0.0 ^{ac}	9±0.5 ^{a}	10±0.5 ^{a}	8±0.5 ^{a}
	25	8±1.0 ^{a}	8±1.0 ^{b}	8±0.0 ^{a}	8±0.5^{ab}	9±0.5 ^{a}	9±0.0 ^{a}	8±0.5 ^{a}
	12.5	8±0.0 ^{a}	8±0.5 ^{a}	8±0.5 ^{a}	7±0.5 ^{a}	9±0.0	8±0.5 ^{a}	8±0.0 ^{a}
Positive control	CIP (30µg/l)	32±0.5 ^{ab}	30±0.5 ^{ac}	30±0.5 ^{ab}	35±0.5 ^{ac}	38±0.5 ^{ab}	36±0.5 ^{ac}	35±0.5 ^{ab}

Negative control	DMSO (10 %)	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	1.0±0.0[a]

Note: Values represent the means (to the nearest whole number) ± standard error of mean (SEM) of triplicate observations. conc. = concentration; CIP = Ciprofloxacin; DMSO = Dimethyl sulfoxide, Superscripts of the same letter in a row are not significantly different at $p \leq 0.05$. n = 3

The antibacterial activity of hot water extract of *J. tanjorenis*is shown inTable 3 D. The antibacterial activities of the hot water extract of *J. tanjorenis* bark, stem, and leaf have activities on almost all the bacterial isolates tested at different concentrations. *P. aeruginosa* had the highest IZD of 39 mm when challenged with 100 mg/ml of the leaf extract of *J. tanjorenis.* The bark, stem, and leaf extract of *J. tanjorenis* significantly ($p < 0.05$) inhibited all the pathogens except *P. aeruginosa and S. aureus.*

Table 3 D. Mean inhibition zone diameter of hot water of extract of *J. tanjorenis* against Gram-positive and Gram-negative bacteria

Crude extract	**Conc. (mg/ml)**	**Inhibition Zone Diameter, IZD (mm)**						
		P. aeruginosa	***S. aureus***	***Proteus sp***	***Klebsiellasp***	***S. pyogenes***	***S. pneumoniae***	***E. coli***
Bark	100	15.±1.0[ab]	12±0.5 [a]	14±1.0 [a]	30±0.0 [a]	32±1.0[ab]	35±1.0[ab]	30±0.0 [a]
	50	13±0.0 [a]	10±0.0 [a]	13±0.0 [a]	30±0.0 [b]	32±0.5[ab]	34±0.5 [a]	28±0.5 [a]
	25	12±1.0[ab]	10±0.5 [a]	10±0.5 [a]	30±0.5 [a]	31±0.0 [b]	33±1.0[ab]	28±0.0 [a]
	12.5	10±0.5[a]	8±1.0 [a]	10±0.5 [a]	30±1.0 [a]	26±0.5 [a]	22±0.0 [a]	28±1.0 [a]
Leaf	100	12±0.0[ab]	35±0.5[ab]	34±1.0[ab]	37±0.0 [b]	35±0.5 [b]	32±1.0	34±0.5 [c]
	50	12±1.0 [c]	30±0.0 [b]	30±0.5 [b]	30±0.5 [a]	28±0.0[ab]	31±0.5 [a]	30±0.0[ab]
	25	10±0.5 [b]	30±1.0 [c]	28±0.5[ab]	30±1.0[ab]	28±0.5[ab]	21±0.0 [a]	29±0.5 [a]
	12.5	8±1.0 [a]	21±0.5 [a]	22±0.0 [a]	28±0.5 [a]	22±0.0 [a]	20±0.5 [a]	29±0.0 [a]
Stem	100	12±0.0 [b]	8±1.0 [a]	34±1.0 [b]	35±0.5 [a]	32±1.0[ab]	29±0.0 [a]	30±0.5 [c]
	50	9±1.0[ab]	8±0.0 [a]	32±1.0[ab]	32±0.0 [a]	32±0.5 [a]	28±0.5 [a]	28±1.0[ab]
	25	9±0.0 [a]	8±0.5 [a]	28±0.5 [a]	30±0.5 [a]	30±0.0 [a]	26±0.5 [a]	28±0.0 [a]
	12.5	8±0.5 [a]	8±0.0 [a]	25±0.5 [a]	30±0.0 [b]	25±0.5 [a]	19±0.0 [a]	25±0.5 [a]
Positive control	CIP (30µg/l)	32±0.5 [ab]	30±0.5 [ac]	35±0.5 [ab]	30±0.5 [ac]	38±0.5 [ab]	33±0.5 [ac]	36±0.5 [ab]
Negative control	DMSO (10 %)	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]

Note: Values represent the means (to the nearest whole number) ± standard error of mean (SEM) of triplicate observations. conc. = concentration; CIP = Ciprofloxacin; DMSO = Dimethyl sulfoxide, Superscripts of the same letter in a row are not significantly different at $p \leq 0.05$. n = 3

Mean inhibition zone diameter of methanol extracts of *A. digitata* and *J. tanjorensis* against Gram-positive and Gram negative bacteria

Table 4 A – B shows the mean inhibition zone diameter of methanol extracts of *A. digitata* and *J. tanjorensis* against Gram-positive and Gram negative bacteria.The methanol extract of *A.digitata* exhibited higher IZDs pathogen tested (Table 4 A). At 100 mg/ml, *Proteus* spp has the highest IZD of 45 mm of the bark fractions, while *S. aureus*, and *S. pyogenes* have 42 mm against the stem subfraction. *P. aeruginosa* showed significant ($p < 0.05$) susceptibility with IZD of 42, to the methanol extract of *A. digitata* leaf. Table 4 B showed the mean inhibition zone diameter of methanol extract of *J. tanjorenis* against Gram-positive and Gram negative bacteria.The methanol extract of *J. tanjorenis* bark at 100 mg/ml showed that *S. aureus* had the highest IZD of 35 mm at 100 mg/ml for the methanol extract of *J. tanjorensis* bark andstem.

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Table 4 A. Mean inhibition zone diameter of methanol extract of *A. digitata* against Gram-positive and Gram negative bacteria

Crude extract	Conc. (mg/ml)	Inhibition Zone Diameter, IZD (mm)						
		P. aeruginosa	***S. aureus***	***Proteus sp***	***Klebsiellasp***	***S. pyogenes***	***S. pneumoniae***	***E. coli***
Bark	100	35±0.0^{b}	40±1.0 ^{b}	44±0.0^{ab}	42±0.5 ^{b}	38±0.0^{ab}	37±0.0 ^{ac}	38±0.5 ^{a}
	50	32±0.0^{ab}	40±1.0 ^{a}	40±1.0 ^{b}	40±1.0^{ab}	37±1.0^{ab}	33±1.0^{ab}	37±1.0^{ab}
	25	31±0.5 ^{a}	40±0.0 ^{a}	40±1.0 ^{a}	30±0.5 ^{a}	35±0.0 ^{b}	30±0.0 ^{a}	35±0.5 ^{a}
	12.5	31±1.0^{a}	40±1.0 ^{a}	40±0.5 ^{a}	30±1.0 ^{a}	26±1.0 ^{a}	30±1.0 ^{a}	31±1.0^{ab}
Leaf	100	42±0.0^{ab}	40±0.0 ^{b}	35±1.0 ^{ac}	40±0.5 ^{a}	25±1.0	30±0.0	35±0.5 ^{a}
	50	37±0.5 ^{a}	40±1.0 ^{a}	35±0.5^{ab}	40±1.0^{ab}	20±0.5	27±0.5	34±1.0^{ab}
	25	33±0.0 ^{a}	30±1.0 ^{a}	30±1.0 ^{a}	40±0.5 ^{a}	20±1.0	20±1.0^{ab}	32±0.5 ^{a}
	12.5	27±1.0 ^{a}	25±0.5 ^{a}	30±1.0 ^{a}	40±1.0^{ab}	19±0.5 ^{a}	19±0.0 ^{a}	27±1.0 ^{a}
Stem	100	40±0.0 ^{a}	42±1.0 ^{ac}	38±1.0^{ab}	35±0.5 ^{a}	42±0.0 ^{a}	35±1.0^{ab}	38±0.5 ^{a}
	50	37±0.5^{ab}	38±0.0 ^{a}	38±0.0^{ab}	35±1.0^{ab}	41±1.0^{ab}	35±0.5 ^{a}	29±1.0^{ab}
	25	34±0.0 ^{a}	35±1.0^{ab}	36±0.5 ^{a}	35±0.0 ^{a}	33±1.0 ^{a}	30±1.0^{ab}	29±1.0^{ab}
	12.5	23±0.5 ^{a}	35±0.0 ^{a}	35±0.0 ^{a}	35±1.0^{ab}	30±0.5 ^{a}	28±0.0 ^{a}	22±0.5 ^{a}
Positive control	CIP (30µg/l)	44±0.5 ^{ab}	42±0.5 ^{b}	45±0.5^{ab}	45±0.5 ^{a}	44±0.5 ^{ab}	40±0.5 ^{ab}	40±0.5^{ab}
Negative control	DMSO (10 %v/v)	0.0±0.0 ^{a}	0.0±0.0^{a}	0.0±0.0 ^{a}	0.0±0.0 ^{a}	0.0±0.0 ^{a}	0.0±0.0 ^{a}	0.0±0.0^{a}

Note: Values represent the means (to the nearest whole number) ± standard error of mean (SEM) of triplicate observations. conc. = concentration; CIP = Ciprofloxacin; DMSO = Dimethyl sulfoxide, Superscripts of the same letter in a row are not significantly different at $p \leq 0.05$. n = 3.

Table 4 B. Mean inhibition zone diameter of methanol extract of *J. tanjorenis* against Gram-positive and Gram negative bacteria

Crude extract	Conc. (mg/ml)	Inhibition Zone Diameter, IZD (mm)						
		P. aeruginosa	*S. aureus*	*Proteus sp*	*Klebsiellasp*	*S. pyogenes*	*S. pneumoniae*	*E. coli*
Bark	100	25±0.5^{ab}	35±0.0^{ab}	22±1.0 ^{ac}	18±1.0 ^{b}	8±1.0 ^{a}	9±1.0 ^{a}	17±0.0 ^{b}
	50	22±1.0^{ab}	30±0.0^{ab}	22±0.5^{ab}	16±1.0^{ab}	8±0.0 ^{a}	9±0.0 ^{a}	16±1.0 ^{a}
	25	17±0.0 ^{b}	28±0.5 ^{a}	20±1.0 ^{b}	12±0.5 ^{a}	8±1.0 ^{a}	9±1.0 ^{a}	16±0.0 ^{a}
	12.5	15±1.0^{a}	28±0.0 ^{a}	17±0.5 ^{a}	10±1.0 ^{a}	8±0.0 ^{a}	9±0.5 ^{a}	14±1.0 ^{a}
Leaf	100	22±1.0^{ab}	32±0.5	19±0.0	21±1.0 ^{ac}	8±1.0 ^{a}	9±1.0 ^{a}	9±0.5 ^{a}
	50	20±0.5 ^{a}	20±1.0	11±1.0	19±0.0^{ab}	8±0.0 ^{a}	9±0.5 ^{a}	9±1.0 ^{a}
	25	20±1.0^{ab}	8±0.5	10±0.5	10±1.0 ^{b}	8±0.5 ^{a}	8±0.0 ^{a}	9±1.0 ^{a}
	12.5	19±0.0 ^{a}	8±1.0	9±0.0	10±0.5 ^{a}	8±0.0 ^{a}	8±1.0 ^{a}	9±0.0 ^{a}
Stem	100	25±0.5 ^{ac}	35±0.0 ^{ac}	30±0.0 ^{ac}	10±0.0 ^{a}	9±0.0 ^{a}	10±1.0 ^{b}	11±1.0^{ab}
	50	20±0.0^{ab}	30±1.0^{ab}	19±0.0^{ab}	9±1.0 ^{a}	9±1.0 ^{a}	8±1.0 ^{a}	10±0.0 ^{a}
	25	20±0.0^{ab}	22±0.5 ^{b}	19±0.0^{ab}	8±0.0 ^{a}	9±1.0 ^{a}	8±0.0 ^{a}	9±1.0 ^{a}
	12.5	17±0.5 ^{a}	8±0.0 ^{a}	11±0.0 ^{a}	8±0.5 ^{a}	9±1.0 ^{a}	8±1.0 ^{a}	9±0.0 ^{a}
Positive control	CIP (30µg/l)	40±0.5 ^{ab}	38±0.5 ^{ac}	35±0.5 ^{ab}	25±0.5 ^{ac}	20±0.5 ^{ab}	25±0.5 ^{ac}	20±0.5 ^{ab}
Negative control	DMSO (10 %)	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}

Note: Values represent the means (to the nearest whole number) ± standard error of mean (SEM) of triplicate observations. conc. = concentration; CIP = Ciprofloxacin; DMSO = Dimethyl sulfoxide, Superscripts of the same letter in a row are not significantly different at $p \leq 0.05$. n = 3

The effects of the combined extracts of *J. tanjorensis* and *A. digitata* on bacteria isolates.

Table 5indicates the minimum bactericidal concentration (MBC) of the combined extracts of methanol extracts of *A. digitata* bark and hot water extracts of *J. tanjorensis* stem. It shows that the MBC of the extract was greater than 100 mg/ml.

Table 5. Synergistic effect of methanol extracts of *A. digitata* bark and hot water extracts of *J. tanjorensis*stem

Organism	MBC of the MBA + HSJ (mg/ml)			
	100	**50**	**25**	**12.5**
P. aeruginosa	+	+	+	+
S. aureus	+	+	+	+
Proteus sp	+	+	+	+
Klebsiellasp	+	+	+	+
S.pyogenes	+	+	+	+
S. pneumonia	+	+	+	+
E. coli	+	+	+	+

Note: *A. digitata*bark (MBA) and hot water extracts of *J. tanjorensis*stem (HSJ); + = Growth; - = No growth

DISCUSSION

The aqueous and methanol extract of the various parts of *A. digitata* and *J. tanjorensis* extracts showed varying degrees of antibacterial activities against the different bacterial pathogens tested. Interestingly, methanol extract of the various fractions possesses the highest antibacterial activities while hot water extracts exhibited hiher activities and cold water extracts gave the least activity for all the extracts tested.

This study showed that methanol extract of *A. digitata*bark gave the highest yield at 75 % followed by methanol extract of *J. tanjorensis* leaf which yielded 70 %. The high yield of *A. digitata* and *J. tanjorensis* methanol extract could be due to its polarity as an extraction solvent. Similarly, the hot water extract of *J. tanjorensis* bark and hot water extract of *A. digitata* bark gave a percentage yields of 66 % and 65 % respectively. However, lowest yield was observed with the cold water extract of *A. digitata* bark at 33 % yield. This shows that the proportion of water-insoluble compounds and water-soluble compound for the *A. digitata* and *J. tanjorensis* varies proportionately depending on the extractant (solvent) or part of the plant extracted.

In this study, the qualitative phytochemical analysis indicated that the aqueous and methanol extracts of different parts of *A. digitata* and *J. tanjorensis* contain saponin, tannin, flavonoid, glycoside, terpenoid, phenol, and alkaloid. This is in agreement with the study carried out by Daniyan*et al.*, (2018); Datsugwai and Yusuf (2017) who also reported similar results. The aqueous extract of *J. tanjorensis*leaf lacked glycoside but was rich in alkaloids and terpenoids. The methanol extract of *J. tanjorensis* leaf proved to be rich in terpenoid and tannin. The aqueous extract of *J. tanjorensis* stem was abundant in flavonoids and alkaloids but lacked glycoside and phenol. The methanol extract of *J. tanjorensis* stem was rich in tannin, flavonoid, and terpenoid but did not have phenol. However, the result of the present study is not in agreement with the work of Viswanathan*et al.*, (2012) where phenols were present in the methanol extracts of *J. tanjorensis.* The aqueous extract of *J. tanjorensis*bark was rich in alkaloids but lacked phenol. The methanol extract of *J. tanjorensis* bark was abundant in tannin, flavonoid, and terpenoid. It indicated that the aqueous extract of *A. digitata* leaf is abundant in flavonoids and alkaloids but lacks glycoside. The methanol extract of *A. digitata* leaf was rich in terpenoid and phenol. The aqueous extract of *A. digitata*stem was abundant in alkaloids. The methanol extract of *A. digitata* stem was rich in tannin, terpenoid, and phenol. The aqueous extract of *A. digitata* bark was rich in phenol and alkaloid. The methanol extract of *A. digitata* bark was rich in tannin, terpenoid, and phenol but lacked flavonoids. This is in agreement with the work done by Abdallah and Ali (2019), which showed that flavonoids were absent in the bark extracts of *A. digitata.*

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The cold water extract *of A. digitata* showed the least inhibition compared to other extracts against all thepathogensat the various concentrations tested. Abdallah and Ali (2019) observed that the aqueous of *A.digitata*were ineffective on *E. coli.* Similarly, Kamatou *et al.* (2011); Ajiboye *et al.* (2020) reported that*A. digitata*extract was not active against *P. aeruginosa, S. aureus,* and *E. coli* while *Klebsiella pneumoniae* showed the lowest inhibition zone at 200 mg/ml of *A. digitata*stem bark. This is in line with the work of Abiona *et al.,* (2015) where it was stated that as the concentration increased, so did the zone of inhibition and vice versa. Thehot water extract of *A. digitata* leaf, bark, and stem had higher antibacterial activity against *S. aureus, Proteus* spp, *Klebsiella*spp at concentrations of 100 mg/ml. The hot water extract of *A. digitata* stem gave the highest IZD of 40 mm against *P. aeruginosa*. This result portends a high antibacterial effect of hot water extract of *A. digitata against P. aeruginosa*. In a similarly study, Ajiboye*et al.,* (2020) observed that the aqueous extract of *A. digitata* gave a high zone of inhibition against *S. aureus.* The methanol extract of *A. digitata* and *J. tanjorenis* gave the highest IZDs at 100 mg/ml for *Proteus* spp (40 mm) and 35 mm for *S. aureus*. Similarly, combined *A.digitata* and *J. tanjorenis* extract in the MBC study showed that the extractshave greater antibacterial activities at concentration not lesser than 100 mg/ml.

CONCLUSION

This study demonstrated that the parts of the two plants used are potentially good sources of antibacterial agents and indicates the significance of these plants in medicine and in assisting primary health care in this part of the world.

Conflict of interest: The authors declare that no conflict of interest exists.

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