Bioactivity of *Khaya Senegalensis* (Desr.) A. Juss. *and Tamarindus indica* L. Extracts Against Selected Pathogenic Microbes

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Abstract : *Khaya senegalensis* and *Tamarindus indica* are widely used medicinal plant in many parts of Africa to manage infectious diseases. The barks of these plants were screened for their phytochemical and their bioactivity activity against some clinical pathogenic microorganisms. Results of the phytochemical screening showed that the aqueous, methanolic and ethanolic extracts of the barks of the plants contain saponins, tannins, basalms, glycosides, steriods, terpenoids and flavonoids. The antimicrobial susceptibility test showed that bark extracts of *K. senegalensis* and *T. indica* had varying inhibition strength on the test organisms. The antimicrobial susceptibility test showed that bark extracts of *K. senegalensis* and *T. indica* had varying inhibition strength on the test organisms. *K. senegalensis* extract were able to inhibit *K. pneumonia, Pseudomonas* 27853 as well as FT3 Feacal. The highest ZOI (19.7mm) was recorded for ethanolic extract on *K. pneumonia* while aqueous extract had the highest ZOI (19.0mm) on FT water. Generally, methanolic extract of *K. senegalensis* conferred higher inhibitory effects on Pseudomonas while the ethanolic extract was best in inhibiting *E. coli* (Carbapenem-resistant strain). All the *T. indica* extracts inhibited *K. pneumonia* and FT water while aqueous and methanol inhibited MRSA. Highest ZOI of 19.7mm was recorded for ethanolic extract on *K. pneumonia* while crude extract had the highest (19.0mm) ZOI on FT water. The minimum inhibitory concentration of the bark extracts ranged from 400mg/ml to 25mg/ml. There was no minimum bactericidal concentration observed from the extracts. Antibiotics susceptibility test using standard antibiotics indicated multi-drug resistance by the test organisms which Ceftriaxone (CRO) 30µg and Cefepime (FEP) 30µg elicited inhibitory activity against *Carbapenem-resistant pseudomonas species, P. aerigunosa ATCC 27853,* and *E. coli (non- carbapenem strain)* respectively. The result of this study, therefore suggest the possibility of using *K. senegalensis* and *T. indica* extracts as antimicrobial agents, which can be a great asset to drug development for purpose of health care delivery in Nigeria.

Keywords: Pathogenic microbes; Antimicrobial; MIC; MBC; Zone of inhibition; Antibiotics.

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1. Introduction

Higher plants and shrubs are believed to contain phytochemical with medicinal values and their secondary metabolites are increasingly harnessed for disease management and curative purposes (Mukiama. 2005). Nearly 30% or more of the modern pharmacological drugs, homeopathic and Ayurvedic medicines are derived directly or indirectly from plants (Nascimento. 2000). Performance-enhancing drugs such as Atropine, Ephedrine and Morphine are few examples of plants derived medicines via folkloric information (Abdullah and Abdullah. 2016).

Medicinal plants offer alternative remedies having contained a wide variety of secondary metabolites such as tannins, saponins, terpenoids, alkaloids and flavonoids. The metabolites are phytochemicals that occur naturally in plants. Some are responsible for color and organoleptic properties and exhibit in-vitro antimicrobial properties (Phillipon and Anderson. 2000). There is growing cases of multidrug-resistant bacterial infections and this continues to pose challenge to public health officials, care givers and general population all over the world (Andran. 2012). A direct consequence of multidrug-resistant infections is the high cost of infectious disease management and re-occurrence of previously contained diseases. Statistics showed that anti-bacterial resistance contributes to the high incidences of acute and chronic infection cases in many Sub-Saharan African countries (Olajuyigbe and Afolanyan. 2012). Medicinal plants offer alternative in curbing spread of diseases since plant extracts possess health benefits. Aqueous and solvent extracts of plants offer anti-inflammatory, antibacterial, anti- carcinogenic and anti-oxidants properties (Cai et al., 2004). Crude and refined extracts of plants could kill or inhibit the growth of hitherto multi-drug resistant pathogenic bacteria and other microorganisms (Ugho et al., 2014).

Information about resistant effects of extracts of *K. Senegalensis* and *T. indica* barks against clinical pathogenic micro-organisms remained relevant with respect to the development of treatments for infectious diseases. Therefore, the objective of this study was to conduct a phytochemical screening and investigate the antimicrobial effects of *K. Senegalensis* and *T. indica* barks against selected clinical pathogenic micro-organisms.

2. Materials and Methods

2.1. Collection, Identification and preparation of Plant materials

Fresh barks of *K. senegalensis* and *T. indica* species were collected from Mokwa, Niger State and authenticated at the Herbarium of the Department of Plant Biology, University of Ilorin, Nigeria. Barks were prepared via washing, shade drying and pulverized using pestle and wooden mortar. The powdered plant material was sieved with fine mesh and kept in plastic bags prior to extraction.

2.2. Extraction Process

One hundred grams of each of the powdered bark of the plant was percolated in 500 ml of methanol, ethanol and distilled water, placed on the rotator shaker for 48 hours at 150 rpm and filtered using muslin cloth. The filtrates were concentrated at 45 °C using a water bath.

2.3. Qualitative Phytochemical Analysis

Chemical tests for the screening and identification of bioactive chemical constituents present in the plants under study were carried following the standard procedures as described by Trease and Evans (1989) and Tor-Anyiin et al. (2011).

2.4. Test Organisms

The test organisms: *Klebsiella pneumoniae, Pseudomonas aerigunosa ATCC 27853, methicillin-resistant Staphylococcusa aureus ATCC 4330* (MRSA), *Escherichia coli ATCC 25922, Carbapenem-resistant Pseudomonas Species* **(**S6 Pseudomonas**)**, *Escherichia coli* (FT3 Feacal) *(Carbapenem resistant strain), Escherichia coli* (FT water) *(Non-carbapenem-resistant strain*) were obtained from the stock culture of the Microbiology laboratory of University of Ilorin, Ilorin, Nigeria and authenticated using cultural and morphological identification, microscopy after Gram's staining as well as biochemical characterization of test organism and maintained in a nutrient agar slant in a refrigerator for future use.

2.5. Preparation and standardization of bacterial inoculum

Bacterial inoculum was standardized using McFarland's standards. This was carried out by picking test organisms growing as pure culture in McCartney bottles and transferring into 10 ml of distilled water in test tubes. Growing media was prepared according to the manufacturer's specification. The media were sterilized at 121°C for 15 min using autoclave.

2.6. Antimicrobial Susceptibility Test (AST)

The antimicrobial screening was carried out using the agar well diffusion method as described by Lino and Deogracious (2006) with slight modifications. The test bacteria were first cultivated in nutrient broth at 37°C for 18 h. Each of the cultures was then adjusted to 0.5 McFarland turbidity standards and inoculated (0.2 ml each) onto Muller Hinton agar (MHA, Oxoid) plates (diameter: 15 cm). A sterile cork borer was used to bore six wells (6 mm diameter). The plates were allowed to stand for 30 min to allow extract to diffuse into the agar then incubated at 37°C overnight. Bacterial inhibition zone was measured (Lino and Deogracious. 2006).

2.7. Minimum Inhibitory Concentration (MIC) of plant extracts and test organisms

MIC of the extracts were determined for each of the test organisms in triplicates at 400 mg/ml, 200 mg/ml, 100 mg/ml, 50 mg/ml, and 25 mg/ml. 1 ml of nutrient broth was added and then a loopful of the test organism previously diluted to 0.5 McFarland turbidity standard was introduced to the test tubes. All test tubes were incubated at 37°C for 24 h (Adetitun et al., 2013).

2.8. Determination of Minimum Bactericidal Concentration

The minimum bactericidal concentration (MBC) was determined by the method of Roimi et al. (1988). All the tubes that showed no microbial growth (no turbidity) after 24 h of incubation was subculture onto the surfaces of freshly prepared Mueller-Hinton agar and incubated at 37°C for another 24 h. MBC was regarded as the lowest concentration of the extract that did not permit a visible bacterial growth on the Mueller Hinton agar after bacterial 24 h incubation. After incubation the plates from which there was no single colony of bacteria was taken as MBC (Ajaiyeoba et al., 2003).

2.9. Statistical analysis

To show the differences in the Zone of inhibition of the extracts against the selected clinical organisms, analysis of variance (ANOVA) was performed. Significant differences between means were determined by Duncan's multiple range tests.

3. Results and Discussion

3.1. Antimicrobial sensitivity

The crude extracts of *K. senegalensis* showed profound inhibition against *Pseudomonas, K. Pneumonia and E. coli (Carbapenem-resistant strain).* There were variability in the growth inhibition among the solvents which may be due to the difference in their polarities. The aqueous and methanolic extracts of *K. senegalensis* had the same inhibitory effects on *K. Pneumonia* with zones of inhibition (ZOI) of 17.7mm compared to ethanolic extract (14.3mm) (Table 1). *K. senegalensis* extract were able to inhibit K. pneumonia, Pseudomonas 27853 as well as FT3 Feacal with no zones of inhibition for S4 E. coli, E. coli ATCC 25922, S6 Pseudomonas, MRSA and FT water. The highest ZOI (19.7mm) was recorded for ethanolic extract on *K. pneumonia* while aqueous extract had the highest ZOI (19) on FT water (Table 1).

Generally, methanolic extract of *K. senegalensis* conferred higher inhibitory effects on *Pseudomonas* while the ethanolic extract was best in inhibiting *E. coli (Carbapenem-resistant*

strain). This finding is similar with the findings of Kubmarawa et al. (2008) and Ugoh et al. (2014). Similarly, all the *T. indica* extracts inhibited *K. pneumonia* and FT water while Aqueous and methanol inhibited MRSA. Highest ZOI of 19.7mm was recorded for ethanolic extract on *K. pneumonia* while crude extract had the highest (19.0mm) ZOI on FT water. The ethanolic extract was not able to inhibit MRSA. However, S4 *E. coli*, *E. coli* ATCC 25922, Pseudomonas 27853, S6 Pseudomonas and FT3 Feacal were resistant to all the *T. indica* extracts (Table 1). Abalaka et al. (2016) reported higher activities of *K. senegalensis* for the root while the leaf had the least extract activity against the selected bacterial and fungal isolates. However, Makut et al. (2008) had a contrary result. They reported that *K. senegalensis* bark extract was not able to inhibit E. coli but had profund ZOI on *S. aureus, S. feacalis* and *C. albicans*. Makut et al. (2008) had similar findings for S. aureus; however, both methanolic and ethanolic extracts were not able to inhibit *E. coli*

Table 1: Antimicrobial effects of aqueous, methanolic and ethanolic barks of *K. senegalensis* and *T. indica* extracts on Clinical micro-organisms

Test Organism	Zone of Inhibition					
	K. senegalensis			*T. indica*		
	Aqueous	Methanol	Ethanol	Aqueous	Methanol	Ethanol
K. pneumonia	17.7^{a}	17.7^{a}	14.3^{b}	12.3^{bc}	13.7^{b}	19.7^{a}
S4 E. coli	N	N	N	N	N	N
E. coli ATCC 25922	N	N	N	N	N	N
Pseudomonas 27853	19.3^{ab}	21.7^{a}	20.3^{a}	N	N	N
S6 Pseudomonas	N	N	N	N	N	N
MRSA	N	N	N	12.3^{a}	12.7^{a}	N
FT3 Feacal	17.3^{b}	17^{b}	19.3^{a}	N	N	N
FT water	N	N	N	19.0^{a}	18.0^{a}	16.7^{b}

Mean with the same superscript along a row are statistically similar

Key: N = no zone of inhibition

There were statistical difference in the ZOI exhibited by the two plant extracts. However, *K.pneumonia* and FT3 water had similar ZOI for *K. senegalensis* aqueous and methanolic extracts while Pseudomonas 27853 had similar ZOI for methanolic and ethanolic extracts. Likewise, ZOI

for *T. indica* were similar for aqueous and methanolic extracts against MRSA and FT water. Escalona-Arranz et al. (2010) and Meléndez (2006) reported similar findings for *S. aureus* and *P. aeruginosa* where *T indica* leaves showed maximum ZOI against *S. aureus* and *P. aeruginosa* at 17.3µ1.5mm and 24.66µ.51mm respectively while Ciprofloxacin and Gentamicin used as standard for the respective organisms had ZOI of 36.0µ µ2.0mm and 37.0 0µ1.73mm. Kubmarwa et al. (2008) reported 14.2mm, 14.3mm and 16.5mm ZOI for aqueous extract on *S. aureus, E. coli* and *P. aeruginosa* 8.4mm, 16.3mm and 18.4mm ZOI were reported for ethanolic extract respectively. Meher and Dash (2013) reported similar sensitivities for *E. coli, P. aeruginosa* and *K. pneumonia* with lower MIC values.

3.2. MIC and MBC of the Selected Plants Crude Extracts

3.2.1. Minimum Inhibitory and Bactericidal Concentration of *K. senegalensis* Extracts

There were variations in the minimum inhibitory concentrations exhibited by the plant extracts on the growth response of selected microbes. *Pseudomonas 27853, K. pneumonia* and *E. coli (Carbapenem-resistant strain)* were susceptible to varying concentrations of *K. senegalensis* crude *extract*. However, there was no corresponding MBC across all solvents used for extraction (Table 1). The varied activities of different extracts of *K. Senegalensis* could be due to the polarity of the solvents which tend to influence the kind of bioactive compound released from the plant material (Altemimi et al., 2017).

Table 2: Minimum inhibitory and bactericidal concentration of *K. senegalensis* extract

Test organisms	Concentration of aqueous extract (mg/ml)					
	400	200	100	50	25	MBC
K. pneumonia	-	-	-	+	+	+
Pseudomonas 27853	-	-	-	-	-	+
E. coli (Carbapenem-resistant strain)	-	-	-	+	+	+
Test organisms	Concentration of ethanolic extract (mg/ml)					
	400	200	100	50	25	MBC
K. pneumonia	-	-	+	+	+	+
Pseudomonas 27853	-	-	-	-	-	+
E. coli (Carbapenem-resistant strain)	-	-	-	-	-	+
Test organisms	Concentration of methanolic extract (mg/ml)					
	400	200	100	50	25	MBC
K. pneumonia	+	+	+	+	+	+
Pseudomonas 27853	-	-	-	-	+	+
E. coli (Carbapenem-resistant strain)	+	+	+	+	+	+

MIC: (-) No Growth or Not Turbid; (+) Growth or Turbid
MBC/MFC: (+) No killing effect; (-) Killing effect

3.2.2. Minimum Inhibitory and Bactericidal Concentration *T. indica* Extracts

The aqueous and solvent extracts of *T. indica* demonstrated profound inhibitory actions against the test organisms. The MIC values of extracts showed that tolerable concentrations of the extracts could hinder pathogenic microbes growth and development. The extract showed MIC to MRSA 4330, *E. coli (non-carbapenem strain)* and *K. pneumonia* at varied concentration. However, none of the extracts gave minimum bactericidal concentration (Table 2). Different plant extracts have reported exhibiting diverse inhibitory activities against pathogenic organisms and this could be caused by intrinsic and extrinsic conditions (Altemimi et al., 2017).

The variation in the MIC of the two plant materials may be due to the phytochemical composition of the respective extracts and the genetic make-up of each test organisms. Different organisms have been shown to respond differently to different and same concentrations of a specific medicinal plant. Emeruwa (1982) and El-Faraley et al. (1983) reported that agents with low antimicrobial activity against an organism would require high concentrations (MIC) while those with high activity requires low concentrations to either inhibit or totally kill such organism. The antimicrobial activities of medicinal plants have been closely associated with the secondary metabolites obtained from these plants. These metabolites have been shown to have physiological activity against known pathogens. Concentration of these metabolites however, may vary within parts of the plant (Ahmadu et al., 2006).

None of the antibiotics was able to inhibit the growth of MRSA *ATCC 4330*, which *T. indica* bark extracts did, this is an indication that phyto-constituents present in the plant extracts are more potent than the standard antibiotics to exert antimicrobial effect on the organisms (Table 2). It may also be due to the fact that every antibiotic has certain life span regarding its efficacy (Jazet et al., 2007). The standard antibiotics is a refined and purified product, while extracts of herbal medicines are mixtures of various plant constituents and some of which can interfere with antimicrobial activity and are subjected to degradation and decomposition in storage (El-Mahmood and Amey. 2007).

Table 3: Minimum inhibitory and bactericidal concentration of *T. indica* extract

Test organisms	**Concentration of aqueous extract (mg/ml)**					
	400	200	100	50	25	MBC
MRSA 4330	-	-	-	-	-	+
E. coli (Non-carbapenem-resistant strain)	-	-	-	-	+	+
Kleb	-	-	-	-	-	+
Test organisms	**Concentration of ethanolic extract (mg/ml)**					
	400	200	100	50	25	MBC
MRSA 4330	+	+	+	+	+	+
E. coli (Non-carbapenem-resistant strain)	+	+	+	+	+	+
K. pneumonia	+	+	+	+	+	+
Test organisms	**Concentration of methanolic extract (mg/ml)**					
	400	200	100	50	25	MBC
MRSA 4330	-	-	-	-	+	+
E. coli (Non-carbapenem-resistant strain)	-	-	+	+	+	+
K. pneumonia	+	+	+	+	+	+

Key for MIC: (-) No Growth or Not Turbid; (+) Growth or Turbid
Key for MBC/MFC: (+) No killing effect; (-) Killing effect

3.3. Phytochemical Constituents of the Crude Extracts

The bark crude extracts of *T. indica* and *K. Senegalensis* were subjected to phytochemical screening and the result revealed the presence of important phytochemical (Table 3). The phytochemical screening of the aqueous, methanolic, and ethanolic extracts of the samples revealed the presence of saponin, flavonoids, quinone, balsalm and phytosterol among all solvents. Tannins and terpenoids are present in aqueous and ethanolic extracts while chlorogenic acid was

absent in all extracts. Bashir et al . (2020); Abdallah et al. (2016); Kubmarwa et al. (2008) and Makut et al. (2008) detected similar phytochemicals. They detected Tannin, Flavonid, Steroid, cardiac glucosides, saponin and some other phytochemicals in *K. senegalensis* bark aqueous, ethanolic aand methanolic extract.

The phytochemical constituents (tannins, flavonoids, saponin, etc.) are secondary metabolites of plants. They offer defense mechanisms against predation, microbial attacks, insect infestation and herbivores (Mustapha. 2014). The antimicrobial activities of medicinal plants have been closely associated with the secondary metabolites obtained from these plants. These metabolites have been shown to have physiological activity against known pathogens. The demonstration of antibacterial activity against both gram positive and gram-negative bacteria may be indicative of the presence of a broad spectrum antibiotic compounds (Srinivasan et al., 2001). The antibacterial effects of the crude extracts could be due to saponin –saponins could form leather; responsible for wound and skin protection (Ahmadu et al., 2006).

Table 4: Results of the phytochemical screening of the bark crude extracts of *K. Senegalensis* and *T. indica*

Constituents	Aqueous		Ethanol		Methanol	
	K. senegalensis	*T. indica*	*K. senegalensis*	*T. indica*	*K. senegalensis*	*T. indica*
Saponin	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Tanins	+	+	+	+	-	-
Quinone	+	+	+	+	+	+
Chlorogenic acid	-	-	-	-	-	-
Balsam	+	+	+	+	+	+
Cardiac glycosides	+	+	-	-	-	-
Phytosterol	+	+	+	+	+	+
Terpenoid	+	+	+	+	-	-

Key: + = present - = absent

4. Conclusion

The crude extracts of *K. senegalensis* and *T. indica* extract had profound antimicrobial activities. The type of extraction solvent influenced their antimicrobial properties and minimum inhibitory concentration. Important phytochemical (flavonoids, tannins, cardiac glycosides, saponin and steroids) are contained in the extracts which shows *K. senegalensis* and *T. indica* as promising antimicrobial agents. This study partially validate the traditional usage of *K. senegalensis* and *T. indica* in multiple traditional African medicinal systems to treat bacterial infections. It is suggested that more re- search be conducted that will further elucidate and characterized the active components and their mechanism of action with the aim of harnessing its enormous potentials as antimicrobial as well as antioxidant.

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