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Comparative Study on Phytochemicals and Antimicrobial Activity of The Root and Stem Bark of *Annona senegalensis* and *Khaya senegalensis* Plants

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Abstract
Medicinal plants serve as a valuable reservoir for numerous therapeutic agents, largely due to the presence of bioactive secondary metabolites. Ongoing scientific efforts aim to systematically investigate these plants to identify potential antimicrobial compounds capable of suppressing pathogenic growth without adverse effects on the host organism. Cold maceration was carried out on *Annona senegalensis* and *Khaya senegalensis* root and stem bark samples, phytochemical screening of secondary metabolites was also determined, and the results showed the presence of some secondary metabolites like saponin, alkaloids, terpenoids, flavonoids etc. Antimicrobial activity of the extracts was also investigated against some selected organism in various concentrations, the tested organisms are *Escherichia coli*, *Shigella dysentiae*, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Candida albican* and *Aspergillus niger* using agar well diffusion method. It was found that *Khaya senegalensis* stem bark extract showed the highest activity against the tested organisms out of the four extracts, the activity of *Annona senegalensis* extract is more pronounced on *Escherichia coli* bacteria with a mean zone of inhibition of 16.00 mm at 400 mg/ml. From the result obtained, *Khaya senegalensis* extract will be more suitable source of production of drugs that can be used to cure diseases caused by *Escherichia coli* like diarrhea and others.

Keywords: Secondary metabolites, medicinal plants, pathogenic gwoth, adverse effect, cold maceration, phytochemical sceening, antimicrobial activity, *Annona senegalensis* and *Khaya senegalensis*.

1. Introduction

The use of traditional medicine is age long and inherent with prospects and challenges in many cultures in Africa (Fabricant and Fansworth, 2021). Even before the recorded history, medicinal plants had been used in the treatment of variety of ailments of mankind, these herbs had been very useful health remedies over the years and are currently in use in many parts of the world (Fabricant and Fansworth, 2021). Some plant parts are used directly by these traditional medicine practitioners, some are extracted before use or modified and combined before administration, in most cases where they are extracted before application, crude extracts are used (Fabricant and Fansworth, 2021). Therefore, one cardinal challenge to contend with is dosage and the other is cytotoxicity issue in traditional medicine cycle, the use of medicinal plants in the treatment of ailments and diseases dates back to prehistory and developing countries mostly rely on traditional medicines (Kumar et al., 2019). Traditional herbs as healers of diseases are important especially to local communities since they are easily accessible, easy to assemble and affordable to the

people (Kumar et al., 2019). Various diseases including cancer and Alzheimer's disease had been reported to be treated with active compounds from plants (Kumar et al., 2019; Sheeja and Kuttan, 2017). Research on phytochemical analyses of plant extracts in most cases correlate the chemical constituents of the plants with their pharmacological activity (Prachayasittikul et al., 2018). The therapeutic effects of medicinal plants are attributed to the phytochemicals in them including flavonoids, alkaloids, steroids, terpenoids, phenolic acids, tannins, and saponins among others (Nyamai et al., 2016). These secondary metabolites exert antimicrobial activity through different mechanisms (Shimada, 2016). Tannins have been found to form irreversible complexes with proline-rich protein resulting in the inhibition of cell protein synthesis (Shimada, 2016). Herbs that have tannin as their main components are astringent in nature and hasten the healing of wounds and inflamed mucous membrane (Okwu and Okwu, 2014). The biological function of flavonoids includes protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatotoxins and tumors (Okwu, 2014).

Nature has made plants useful throughout the existence of man (Mustapha, 2013). Man uses plants as food, clothing, fuel, shelter and the most useful necessity of life which is the maintenance and management of different diseases, man suffers many diseases and God have provided the cure through the use of plant's roots, seeds, leaves, flowers, berries or bark for medicinal purposes (Mustapha, 2013). Today, most pharmaceutical drugs are derived from excellent ingredients in medicinal plants, in folk medicine, many natural raw drugs have the potential to treat many diseases and disorders, one of which is *Annona senegalensis*; Family: Annonaceae generally known as 'African custard-apple' and usually known as Gwándàn dààjìì (Hausa), dukuu-hi (Fulani) (Mustapha, 2013).

Nigeria has a tropical climate and vegetation with a countless numbers of varieties of plant species, most of these plants could have medicinal uses that are unexploited (Aguoru, 2017). Knowing the chemical composition of the compounds contained in the medicinal plant is a prerequisite to solving a major feat in traditional medicine operation, this could enable drug synthesis from such plants to take place, as a result, some problems relating to dosage, toxicities and antagonisms could be solved (Aguoru, 2017).

2. Materials and Methods

Materials and Equipment's

The equipment used for this experiment included: Volumetric flasks, filter paper, weighing balance, test tubes and test tube rack, measuring cylinders, funnel beakers, and other standard laboratory apparatus.

Chemicals and Reagents

All reagents and chemicals used in this research were of analytical grade. These included: methanol, acetic acid, mercury(II) chloride ($HgCl_2$), ferric chloride ($FeCl_3$), sulphuric acid (H_2SO_4), potassium iodide (KI) and distilled water.

Preparation of Reagents

Dragendorff's Reagent

Dissolve 0.88 g of $Bi(NO_3)_3$ in a mixture of 40 cm^3 distilled water and 10 cm^3 acetic acid. Separately, dissolve 8.0 g of potassium iodide (KI) in 20 cm^3 distilled water. Mix both solutions together in a 250 cm^3 volumetric flask and make up to volume with distilled water.

Mayer's Reagent

Dissolve 1.36 g of mercury(II) chloride ($HgCl_2$) in 40 cm^3 distilled water. Separately, dissolve 5.0 g of potassium iodide (KI) in 20 cm^3 distilled water. Combine the two solutions in a 100 cm^3 volumetric flask and dilute to the mark with distilled water.

10% (w/v) Ferric Chloride Solution

Weigh 10.0 g of ferric chloride ($FeCl_3$). Dissolve in 40 cm^3 distilled water. Transfer the solution quantitatively into a 100 cm^3 volumetric flask and dilute to the mark with distilled water.

10% (w/v) Lead Acetate Solution

Weigh 10.0 g of lead acetate. Dissolve in 40 cm^3 distilled water. Transfer the solution quantitatively into a 100 cm^3 volumetric flask and fill to the mark with distilled water.

1% (v/v) Hydrochloric acid

A 1.0 cm3 of concentrated hydrochloric acid was measured and dissolved in about 100 cm3 volumetric flask. The flask was then filled up with distilled water.

1% (w/v) Barium chloride solution

A 1.0 g of BaCl2 was weighed using beam balance and dissolved in a 100 cm3 volumetric flask and distilled water was added to volume.

1% (v/v) Sulphuric acid

A 1.0 cm3 ¬¬H2SO4 was measured using posture pipette, the concentrated acid was diluted into a 100 cm3 volumetric flask containing 99.0 cm3 of distilled water.

Methods

Sample collection and preparation

Fresh samples of root and stem bark from *Annona senegalensis* and *Khaya senegalensis* were harvested in October 2022 from Bauchi Local Government Area, Bauchi State. The plant species were authenticated at the Department of Biological Sciences, Abubakar Tafawa Balewa University, Bauchi.

Grinding and extraction

The collected plant materials were air-dried and ground into a fine, uniform powder using a mortar and pestle. Approximately 150 g of each powdered sample underwent cold maceration in 300 ml of 80% methanol for 48 hours, following the method described by Okoye (2022). The resulting extracts were filtered, concentrated with a rotary evaporator, and oven-dried at 40 °C to yield the crude methanolic extracts. The percentage yield of the extracts were calculated and subjected to phytochemical analysis using standard methods (Trease and Evans, 2019; Harbone, 2018).

Percentage Yield Calculation

The percentage yield of the extracts was determined using the formula:

$$\text{Percentage Yield} = (\text{Weight of Extract} / \text{Weight of Dried Powdered Sample}) \times 100$$

Preparation of Extract Stock Solution

A quantity of 200 mg of each extract was accurately weighed and dissolved in a small amount of water in a beaker. The solution was then quantitatively transferred into a 100 cm^3 volumetric flask and diluted to the mark with distilled water, resulting in a final concentration of 2 mg/cm^3.

Dilution of the Stock Solution

To prepare a diluted solution, 1.0 cm^3 of the stock solution was measured and transferred into a 100 cm^3 volumetric flask. The volume was made up to the mark with distilled water, yielding a final concentration of 0.02 mg/cm^3 (20 $\mu g/cm^3$).

Phytochemical screening

The solutions of the extracts were subjected to preliminary phytochemical analysis to test for the presence/absence of the various classes of active chemical constituent such as saponins, flavonoids, tannins, alkaloids, terpenoids and cyanogenic glycoside using standard laboratory techniques as reported by Sood et al. (2022) and Chhetri et al. (2018).

Test for Phenolic Compounds (Ferric Chloride Test)

The extracts were dissolved in 10 cm^3 of distilled water. To 2 cm^3 of each extract, a few drops of 2% ferric chloride solution were added. The appearance of a dark green color indicated the presence of phenolic compounds.

Test for Tannins

To 2 cm^3 of each extract, 1 cm^3 of distilled water and three drops of 10% ferric chloride solution were added. The formation of a blue or green-black color confirmed the presence of tannins.

Test for Flavonoids (Lead Acetate Test)

To 1 cm^3 of each extract, a few drops of 10% lead acetate solution were added. The development of a yellow precipitate indicated the presence of flavonoids.

Test for Terpenoids (Salkowski Test)

To 1 cm^3 of each extract, 3 cm^3 of chloroform was added. The mixture was then combined with 2 cm^3 of concentrated sulfuric acid. The presence of terpenoids was confirmed by the appearance of a reddish-brown coloration at the interface.

Test for Glycosides

To 5 cm^3 of each extract, 2 cm^3 of glacial acetic acid containing a drop of ferric chloride solution was added. The mixture was carefully underlayered with 1 cm^3 of concentrated sulfuric acid. The formation of a brown ring at the interface indicated the presence of deoxy sugar of cardenolides.

Test for Alkaloids

To 1 cm^3 of each extract, a few drops of concentrated hydrochloric acid and Dragendorff's reagent were added. The formation of a white precipitate confirmed the presence of alkaloids.

Test for Saponins (Froth Test)

The extracts were diluted with distilled water to a total volume of 20 cm^3 and shaken in a graduated cylinder for 15 minutes. The persistence of foam for 10 minutes indicated the presence of saponins.

Test for Steroids

To 1 cm^3 of each extract, 2 cm^3 of chloroform and a few drops of concentrated sulfuric acid were added along the side of the test tube. The appearance of a red coloration in the lower chloroform layer indicated the presence of steroids.

Preparation of Microbial Inoculums

Microbial cultures were prepared and utilized during the research period. Nutrient Broth (NB) was prepared and dispensed into multiple tubes, followed by sterilization. Pure microbial cultures were collected and inoculated into the tubes using an inoculation needle or loop. The tubes were then incubated at 37°C for 18-24 hours for bacterial growth before being used in the experiments.

Preparation of Nutrient Agar Medium

A 1000 ml batch of nutrient agar medium was prepared, and its pH was adjusted to 6.8 using a pH meter by adding either acid or alkali as needed. The medium was then sterilized in an autoclave at 121°C, under a pressure of 15 lbs for 15 minutes, followed by cooling.

Screening for Antimicrobial Activity (Agar Well Diffusion Method)

Antimicrobial activity was assessed using the agar well diffusion technique described by Idu and Igeleke (2022). Sterile Petri dishes were filled with nutrient agar medium and allowed to solidify. Each microorganism culture was inoculated onto two replicate plates using a sterile swab stick. Wells were created in the medium using a 10 mm cork borer, with four evenly spaced wells on each plate. Different concentrations (50, 100, 200, and 400 mg/ml) of the extracts (1.0 ml each) were introduced into the wells. The plates were incubated at 37°C for 24 hours, after which the zones of inhibition were measured using a ruler. Standard antibiotics, ketoconazole (200 mg/ml) and chloramphenicol (250 mg/ml), were used as positive controls (Idu and Igeleke, 2022).

Determination of Minimum Inhibitory Concentration (MIC):

The MIC of the crude extracts was determined using the broth dilution method. Test tubes were labeled, and 0.2 ml of nutrient broth was added to each, followed by 0.1 ml of inoculum. Varying concentrations of the extracts (50, 100, 200, and 400 mg/ml) were then introduced (0.2 ml per test tube). Control tubes without the extract were included (Andrews, 2021). Uninoculated tubes served as negative controls for sterility checks, while positive controls ensured the medium's suitability for microbial growth and inoculum viability. After mixing thoroughly, the tubes were incubated at 37°C for 24 hours. MIC was determined as the lowest concentration of the extract that inhibited visible microbial growth, indicated by the absence of turbidity (Andrews, 2021).

Determination of Minimum Bactericidal Concentration (MBC):

The MBC was defined as the lowest extract concentration capable of killing 99.9% of the bacterial inoculum after 18-24 hours of incubation at 37°C. To determine the MBC, the contents of tubes showing no growth were subcultured onto extract-free Mueller-Hinton agar plates without antibiotics or extracts and incubated for 24 hours. The absence of bacterial growth confirmed the bactericidal effect (French, 2016).

3. Results

Percentage yield

The percentage yield of the *Annona senegalensis* stem bark extract was 23.2% while that of the root was 18.7% and that of *Khaya senegalensis* stem bark extract was 37.3% while that of the root was 28.7%

Physical properties

The stem bark and root of *Annona senegalensis* and *Khaya senegalensis* was extracted using 80% aqueous methanol, the table below shows the colour, texture and the quantity obtained of the extracts.

Table 1: Some physical properties of the 80% methanolic crude extract of *Annona senegalensis* and *Khaya senegalensis* root and stem bark.

Plant Sample	Quantity of sample used (g)	Quantity of solvent used (ml)	Quantity of extract obtained (g)	Texture	Colour
ASB	150	300	34.8	Dried	Dark green
ASR	150	300	28.1	Semi-oily	Dark green
KSB	150	300	56.0	Dried	Reddish
KSR	150	300	43.6	Dried	Reddish

KEY: ASB = *Annona senegalensis* stem bark, ASR = *Annona senegalensis* root, KSB = *Khaya senegalensis* stem bark, KSR = *Khaya senegalensis* root.

Phytochemical screening

Phytochemical screening was carried out on the crude extracts of the root and stem bark of *Annona senegalensis* and *Khaya senegalensis* plants for qualitative determination of various secondary metabolites and the results obtained were tabulated in the table below:

Table 2: Phytochemical screening of the root and stem bark extracts of *Annona senegalensis* and *Khaya senegalensis*

Secondary Metabolite	ASB Extract	ASR Extract	KSB Extract	KSR Extract
Alkaloids	-	+	-	-
Phenolics	-	-	-	-
Tannins	+	+	+	+
Flavonoids	+	+	+	+
Saponin	+	+	+	+
Terpenoids	+	+	-	-
Glycosides	-	-	+	+
Steroids	-	-	+	+

KEY: + = Presence, - = Absence, ASB = *Annona senegalensis* stem bark, ASR = *Annona senegalensis* root, KSB = *Khaya senegalensis* stem bark, KSR = *Khaya senegalensis* root.

Antimicrobial activity

The antimicrobial activity was determined by the agar disc diffusion method. In this study, *Escherichia coli*, Shigella dysentriae, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Candida albican* and Aspergillus nige were used for assessing the antimicrobial activity. The assessment of antimicrobial activity was based on the measurement of diameter of inhibition zone formed by dissolving the extract in distilled water to form different concentrations. The results of the assessment are summarized in table 3 below.

Table 3: The mean zone of inhibition (mm) of the extracts against some selected organisms from different concentrations

Extract	Conc. (mg/ml)	*Escherichia coli*	*Shigella dysentriae*	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Candida albican*	*Aspergillus niger*
KSB	400	16.00	11.50	13.50	10.00	8.50	0.00
	200	13.00	8.50	9.50	6.00	4.50	0.00
	100	10.00	5.50	4.50	2.50	3.00	0.00
	50	5.00	3.00	2.00	2.00	2.00	0.00
KSR	400	11.00	9.50	8.00	7.50	3.50	0.00
	200	7.00	7.50	5.00	5.50	3.00	0.00
	100	4.50	5.00	3.00	3.00	2.00	0.00
	50	3.00	2.00	2.00	2.00	0.00	0.00
ASB	400	14.00	10.00	9.50	7.00	9.50	2.00
	200	11.00	7.50	7.50	5.00	7.50	0.00
	100	8.00	4.50	4.00	0.00	5.00	0.00
	50	5.00	2.00	2.00	0.00	2.50	0.00
ASR	400	8.00	5.50	3.00	0.00	0.00	0.00
	200	5.00	4.00	0.00	0.00	0.00	0.00
	100	4.00	0.00	0.00	0.00	0.00	0.00
	50	3.00	0.00	0.00	0.00	0.00	0.00
Control	Chloramphenicol (250 mg/ml)	31.50	32.00	29.50	27.00	ketoconazole (200 mg/ml)	
						20.00	18.00

KEY: ASB = *Annona senegalensis* stem bark, ASR = *Annona senegalensis* root, KSB = *Khaya senegalensis* stem bark, KSR = *Khaya senegalensis* root.

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) result obtained from this study revealed that different concentrations of the extracts served as the MIC values against the organisms. Some of the organisms were more sensitive to the extracts even at a low MIC value, while others were only sensitive at a higher MIC value and some other organisms are not sensitive to the extracts at all. The results are summarized in table 4 below.

Table 4: Minimum Inhibitory Concentration (MIC) mg/ml of the extracts of *Annona senegalensis* and *Khaya senegalensis* root and stembark

Extract	*Escherichia coli*	*Shigella dysentriae*	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Candida albican*	*Aspergillus niger*
KSB	100	200	200	200	200	–
KSR	200	200	200	400	400	–
ASB	200	200	200	400	400	–
ASR	400	400	400	–	–	–

KEY: ASB = *Annona senegalensis* stem bark, ASR = *Annona senegalensis* root, KSB = *Khaya senegalensis* stem bark, KSR = *Khaya senegalensis* root.

Minimum Bactericidal concentration (MBC)

The results of the minimum bactericidal concentration (MBC) of the extracts yielded a higher MBC values of mostly 400 mg/ml of some extracts while some organisms might be above 400 mg/ml. The results are summarized in table 5 below.

Table 5: Minimum Bactericidal Concentration (MBC) mg/ml of the extracts of *Annona senegalensis* and *Khaya senegalensis* root and stembark

Extract	*Escherichia coli*	*Shigella dysentriae*	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Candida albican*	*Aspergillus niger*
KSB	200	400	400	400	400	–
KSR	400	400	400	–	–	–
ASB	400	400	400	–	–	–
ASR	–	–	–	–	–	–

KEY: ASB = *Annona senegalensis* stem bark, ASR = *Annona senegalensis* root, KSB = *Khaya senegalensis* stem bark, KSR = *Khaya senegalensis* root.

4. Discussion of Results

Percentage yield

It was observed that, the percentage yield of *Khaya senegalensis* stem bark extract was the highest among the four extracts with a percentage yield of 37.3% which is 56.0 (g) from the dried sample of 150 (g), followed by *Khaya senegalensis* root extract of 28.7% which is 43.6 (g) from 150 (g) of the dried sample, then *Annona senegalensis* stem bark extract of 23.2% which is 34.8 (g) from 150 (g) of the dried sample, then finally *Annona senegalensis* root of 18.7% which is 28.1 (g) from 150 (g) of the dried sample. This is in line with the findings of Ahmed (2020), which reported to have obtained the percentage yield of *Annona senegalensis* stem bark to be 38.6% and *Annona senegalensis* root to be 29.2% of the aqueous extracts by using 100 (g) of the dried sample in 1 liter of the solvent.

Physical properties

Table 1 revealed some physical properties of the four extracts of *Annona senegalensis* and *Khaya senegalensis* root and stem bark. A dark green coloration was observed in the 80% methanolic extracts of the root and stem bark of *Annona senegalensis*, while a reddish coloration was observed in the extracts of both the root and stem bark of *Khaya senegalensis* plant. Three out of the four extracts showed a fully dried texture, while *Annona senegalensis* root extract showed a semi-oily texture which is not a fully dried texture, nor a full oily texture. The quantity of the dried samples used were all 150 (g) which were dissolved in 300 ml of the solvents, and different quantities obtained were determined by the percentage yield which was explained above.

Phytochemical screening

From table 2, we can observe the result of the qualitative phytochemical screening of the 80% methanol extracts of *Annona senegalensis* and *Khaya senegalensis* root and stem bark were presented. The data indicates the presence or absence of eight metabolites that were studied. The results showed that among the eight metabolites, tannin, flavonoid and saponin were positive in all the four extracts, while phenolic is absent in all the four extracts. Alkaloid is present in *Annona senegalensis* extract only, while it is absent in the remaining three extracts. Glycosides and Steroids are present in both root and stem bark extracts of *Khaya senegalensis* while it was found to be absent in *Annona senegalensis*. Finally, terpenoids are present in *Annona senegalensis* root and stem bark extracts, while absent in *Khaya senegalensis* extracts. Similar studies by Aguoru et al. (2017) and Kuta et al. (2015) consistently reported phytochemical constituents of *Khaya senegalensis* to be alkaloids, tannins, saponins and flavonoids. It was also reported from Usman et al. (2017) that saponins, tannins, alkaloids and cardiac glycosides were present in the root bark of *Annona senegalensis*. Therefore, the results of the phytochemical analysis of the crude extracts of *Khaya senegalensis* and *Annona senegalensis* obtained in this study conforms to the previous reports.

Antimicrobial activity

Table 3 shows the antimicrobial activity of *Annona senegalensis* and *Khaya senegalensis* root and stem bark extract, *Khaya senegalensis* stem-bark extract shows the highest activity against the tested organisms, which is more pronounced on *Escherichia coli* (16.00 mm) at 400 mg/ml, followed by *Staphylococcus aureus* (13.50 mm) at 400 mg/ml, then Shigella dysentriae (11.50 mm) at 400 mg/ml, followed by *Streptococcus pneumonia*e (10.00 mm) at 400 mg/ml, finally, *Candida albican* (8.50 mm) at 400 mg/ml, but no observable activity against *Aspergillus niger*. This

is in line with research carried out by Abdallah et al. (2016) were it was reported that ethanolic extracts of the leaf and stem bark of *Khaya senegalensis* were active against some tested organisms of *Escherichia coli*, Shigella and Salmonella due to the presence of some secondary metabolites such as: saponin, flavonoids, alkaloid and tannin.

Annona senegalensis stem bark is the next extract with higher activity against the organisms, which shows a more pronounced activity against *Escherichia coli* with a mean zone of inhibition of 14.00 mm at 400 mg/ml, followed by Shigella dysentriae with a mean zone of inhibition of 10.00 mm at 400 mg/ml, then *Staphylococcus aureus* and *Candida albican* which both showed a mean inhibition zone of 9.50 mm at 400 mg/ml with little differences in the lower concentrations, then followed by *Streptococcus pneumonia*e with a mean zone of inhibition of 7.00 mm at 400 mg/ml, while it shows a very little activity against *Aspergillus niger* with a mean of inhibition of 2.00 mm at 400 mg/ml and no observable activity in the lower concentrations. *Khaya senegalensis* root extract is the next extract with an intermediate activity, it shows it highest activity on *Escherichia coli* with a mean zone of inhibition of 11.00 mm at 400 mg/ml, followed by Shigella dysentriae with a mean zone of inhibition of 9.50 mm at 400 mg/ml, then *Staphylococcus aureus* with a mean inhibition zone of 8.00 mm at 400 mg/ml followed by *Streptococcus pneumonia*e with a mean zone of inhibition of 7.50 mm at 400 mg/ml (which is more than the activity observed in *Annona senegalensis* stem-bark extract) while it shows a low activity on *Candida albican* with a mean inhibition zone of 5.50 mm at 400 mg/ml, and no observable activity against *Aspergillus niger*. It was found in the literature of Kubmarawa et al. (2018) that *Khaya senegalensis* root has antimicrobial activity against *Staphylococcus aureus*, Streptococcus species *Escherichia coli*, Pseudomonas aeruginosa, Salmonella spp. and Baccilus subtilis. These findings Support the claim for its treatment of bacterial and fungal infections (Kubmarawa et al., 2018)

The last tested extract was *Annona senegalensis* root, which shows the least activity against all the four extracts, it shows a medium activity of 8.00 mm mean zone of inhibition on *Escherichia coli* at 400 mg/ml, low activity of 5.50 mm mean zone of inhibition on Shigella dysentriae at 400 mg/ml, and a little activity on *Staphylococcus aureus* with 3.00 mm mean zone of inhibition and no observable activity on *Streptococcus pneumonia*e, *Candida albican* and *Aspergillus niger* in all the prepared concentrations of 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml. Chloramphenicol was used as a positive control for the bacteria with 31.50 mm zone of inhibition on Eschericia coli at 250 mg/ml, 32.00 mm zone of inhibition on Shigella dysentriae at 250 mg/ml, 29.50 mm zone of inhibition on *Staphylococcus aureus* at 250 mg/ml and 27.00 mm zone of inhibition on *Streptococcus pneumonia*e at 250 mg/ml. Ketoconazole of 200 mg/ml was also used as a positive control for the fungi, which it shows a zone of inhibition of 20.00 mm on *Candida albican* and 18.00 mm on *Aspergillus niger*. Distilled water was used as a negative control for all the organisms. The antimicrobial activity of *Annona senegalensis* was reviewed by Johnson and Olatoye (2012) which was reported to have an activity against *Staphylococcus aureus*, Shigella flexneri, Salmonella paratyphi and Pseudomonas aeruginosa (Johnson and Olatoye, 2012).

Minimum inhibitory concentration

Table 4 indicates the minimum inhibitory concentrations of the four extracts, it was revealed that *Khaya senegalensis* stem-bark extract inhibits the growth of the organisms at a minimum concentration of 100 mg/ml (E. coli), while others at 200 ml/ml except *Aspergillus niger* which was found out to be resistant in all the concentrations. *Khaya senegalensis* root and *Annona senegalensis* stem-bark extracts inhibits the growth of *Escherichia coli*, Shigella dysentriae and *Staphylococcus aureus* at 200 mg/ml while *Streptococcus pneumonia* and *Candida albican* at 400 mg/ml, and *Aspergillus niger* is resistant at all concentrations. *Annona senegalensis* root extract inhibits the growth of *Escherichia coli*, Shigella dysentriae and *Staphylococcus aureus* at 400 mg/ml while *Streptococcus pneumonia*, *Candida albican* and *Aspergillus niger* resist inhibition in all the tested concentrations. From the above MIC result, it was clearly understood that *Khaya senegalensis* stem-bark has the highest inhibition effect on the tested organism. This result contradicts the findings of Abdallah et al. (2016) where he reported the MIC values of *Khaya senegalensis* from ethanolic stem bark extract ranged from 12.5 mg/ml to 25.0 mg/ml against *Escherichia coli*, Shigella and *Staphylococcus aureus*. The leaf ethanolic extract ranged from 25.0 mg/ml to 50.0 mg/ml on *Escherichia coli* and Shigella. While both the aqueous leaf and stem bark extracts were less effective with MIC value of 50 mg/ml. (Abdallah et al., 2016). But it is in line the report from Kuta et al. (2015) where the minimum inhibitory concentration

of *Khaya senegalensis* against four bacteria species (*Staphylococcus aureus*, Pseudomonas aeruginosa, *Streptococcus pneumonia* and *Escherichia coli*) was 200 and 400 mg/ml (Kuta et al., 2015).

Minimum bactericidal concentration

Table 5 shows the minimum bactericidal concentration of the four extracts, *Khaya senegalensis* stembark shows lowest MBC value against *Escherichia coli* at 200 mg/ml, while others at 400 mg/ml and *Aspergillus niger* showed no MBC value. *Khaya senegalensis* root and *Annona senegalensis* stem-bark extracts showed MBC value at 400 mg/ml for *Escherichia coli*, Shigella dysentriae and *Staphylococcus aureus* while others were resistant all the tested concentrations. *Annona senegalensis* root extract showed no activity on all the tested organisms at all the concentrations. The above MBC values contradict the findings of Abdallah et al. (2016) where he found the MBC values of *Khaya senegalensis* extracts to be 12.5 mg/ml on *Escherichia coli* and Shigella in stem bark ethanolic extract and also in the leaf ethanolic extract on the same *Escherichia coli* and Shigella to be 25.0 mg/ml, but in aqueous leaf and stem bark extracts MBC were not detected (Abdallah et al., 2016). On the hand, the MCB values obtained conforms to the report of Kuta et al. (2015) where the minimum bactericidal concentration was found to be 400 and 800 mg/ml of *Khaya senegalensis* against *Staphylococcus aureus*, Pseudomonas aeruginosa, *Streptococcus pneumonia* and *Escherichia coli* (Kuta et al., 2015).

5. Conclusion

In summary, the stem bark extract of *Khaya senegalensis* demonstrated the most potent antimicrobial activity among the tested samples, particularly against *Escherichia coli*. This suggests its potential as a promising natural agent for treating *Escherichia coli*-related infections.

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