Phytochemical Analysis and Antimicrobial Activity of Methanolic and Ethanolic Leaves, Barks and Roots Crude Extracts of *Khaya Senegalensis*

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Abstract- The aim of this work was to investigate and compare the phytochemical screening and antimicrobial activities of different crude extracts from leaves, barks and roots of *Khaya senegalensis*. Two different organic solvents including methanol and ethanol were used to prepare the crude extracts from the fresh and dry leaves, barks and roots. Antimicrobial activities of different crude extracts from dry and fresh leaves of *Khaya senegalensis* were determined by agar disc diffusion method with minor modification. *In vitro* phytochemical screening for all crude extracts from all leaves, barks and the roots were tested and shown positive result for all the compounds. The methanol crude extract and its derived fractions from leaves, barks and roots showed small and moderate antibacterial potential with one gram positive (*Staphylococcus aureus)* and three gram negative *(Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa)* bacteria in the range of 0–21%. In conclusion, all organic crude extracts from leaves, barks and roots could be used as potential sources of new antimicrobial properties.

Keywords: *Khaya senegalensis*, Organic solvents, Soxhlet extractor, Phytochemical screening, Antimicrobial activity

I. INTRODUCTION

The study and use herbal medicine has increasingly become a less toxic source of medicinal of plants used for the treatment of many diseases. Native Americans traditionally used about 2500 of the approximately 20,000 plant species that are native to North America. About 80% of the population worldwide use traditional medicine, which has compounds derived from medicinal plant (1, 2). *Khaya senegalensis* (Desr.) A. Juss. is a large meliaceous tree native to the sub-Sahara savannah from Senegal to Uganda, and one of the most popular traditional medicines in Africa. The decoction of the bark is extensively used as febrifuge which could be associated with its use as an antimalarial drug. This genus is a main African mahogany closely related to the South American genus Swietenia, which is one of the main source of rings B, D-seco limonoids such as mexicanolides having a bicyclo[3.3.1]-ring system. Several types of rings B,D-seco limonoids containing mexicanolides and their ring A bridged phragmalin limonoids have been also reported from *Khaya senegalensis* (3). These phytoconstituents comprises alkaloids, flavonoids, phenols, tannins, saponins and sterols. The phytoconstituents of *Khaya senegalensis* were analyzed from various parts of the plant like the leaf root and shoot. The plant finds application in the treatment of diarrhea and skin diseases (4). It is used in the treatment of catarrh, epilepsy, insanity, hysteria, rheumatic pains, hemorrhoids, painful menstruation, skin-ulcers and wounds. It is also used in the treatment of burns. It is used to calm cough and to treat laryngitis and treacheries as well as so many bacterial diseases (5). Plant extracts have greater potential as antimicrobial compounds against microorganisms and that they can be used in the treatment of infectious caused by disease causing pathogens (1).

Several reports have been carried out on plants with antimicrobial activity against bacteria, bacterial pathogens and fungi. Moreover, scientific studies and the results on antimicrobial and phytochemical screening on ethanol and hydro alcoholic crude extracts of this plant have been reported earlier (6). But our study has been planned to determine the antimicrobial activity and phytochemical compounds of different organic crude extracts from both dry and fresh leaves *Khaya senegalensis.* The antimicrobial activity of different crude extracts from both fresh and dry leaves against some selective pathogenic bacteria locally available for possible development of new drugs for the prevention and treatment of infectious diseases caused by bacterial pathogens. Therefore, the aim of this present work is to investigate the phytochemical screening and antimicrobial activities of different crude extracts from dry and fresh leaves of *Khaya senegalensis* (1)

II. MATERIALS AND METHODS

Materials

The chemicals used in this present study such as methanol and ethanol were purchased from Sigma–Aldrich, Germany. Methanol was obtained from Emsure, Germany. Ammonia was obtained from Appli Chem, Germany. Sodium hydroxide and sulphuric acid were obtained from Ohilip Harris, England. Filter papers that were used in the disc were purchased from Whatman, GE Healthcare companies, China. The bacterial strains such as *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E.Coli*), *Klebsiella pneumoniae* (*K. pneumoniae*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) were obtained from Federal Medical Center, Katsina, Nigeria. The UV spectroscopy (UV-1800 Shimadzu spectrophotometer, Japan) was used for measuring the absorbance of the samples.

Plant material

The leaves, barks and roots samples of *Khaya senegalensis* were collected in January, 2014 from the Botanical

garden of the Department of Biology, Umaru Musa Yar' adua University, Katsina, Nigeria and the species were identified at the herbarium unit of the same Department. The herbarium voucher specimen was deposited. The leaves, barks and the roots samples were immediately washed and some fresh parts were immediately soaked into the appropriate organic solvents in a separate containers and the remaining parts were shade-dried for four weeks to constant weights. The dried samples were pounded separately to fine powder using blender, and then stored individually in air-tight containers until the time of extraction.

Extraction procedure for methanolic extract samples

The fresh parts of leaves, barks and roots that were soaked immediately after the plants samples were identified, were left into the methanol for 3 days, after then were filtered for further analysis. The dried leaves, barks and roots powder samples (150 g) were extracted each with methanol solvent (350 ml) in separate flask for 7 days using soxhlet extractor until complete extraction. After extraction, the sample was filtered with filter paper (Whatmann 41). The methanol solvent was evaporated using a rotary evaporator under pressure for 30 min resulting in a semi solid crude extracts (7.2 g, 8.9g and 10.6g). The dried methanol crude extracts (0.97 g, 1.03g, 1.6g) were transferred into test tube for antimicrobial and phytochemical screening.

Extraction procedure for ethanolic extract samples

The fresh parts of leaves, barks and roots that were soaked immediately after the plants samples were identified, were left into the ethanol for 3 days, after then were filtered for further analysis The dried leaves, barks and roots powder samples (150 g) were extracted each with ethanol solvent (350 ml) in separate flask for 7 days using soxhlet extractor until complete extraction. After extraction, the sample was filtered with filter paper (Whatmann 41). The methanol solvent was evaporated using a rotary evaporator under pressure for 30 min resulting in a semi solid crude extracts (6.2 g, 5.9g and 6.6g). The dried methanol crude extracts (0.54 g, 0.43g, 0.83g) were transferred into test tube for antimicrobial and phytochemical screening.

Preliminary phytochemicals screening

The stock solution was prepared from each of the crude extracts such as methanol and ethanol extracts (100 mg); and was dissolved in 10 ml of its own mother solvents. The obtained stock solutions were subjected to preliminary phytochemical screening.

Test for alkaloids

The dry powder samples (1g) were taken in a test tube and an ammonia solution (3 ml) was added to it. They were allowed to stand for few minutes. Then chloroform (10 ml) was added to the test tube samples which was shaken and then filtered to remove the powder samples. The chloroform was evaporated using a water bath and Mayer's reagent (2 ml) was added. A cream colored precipitate was immediately produced which indicates the presence of alkaloids.

Test for flavonoids

A few drops of diluted sodium hydroxide solution were added to the stock solution of *Khaya senegalensis* (0.5 ml). An intense yellow colour appeared in the plant crude extract, which became colorless upon the addition of a few drops of diluted H_2SO_4 acid. This shows the presence of flavonoids.

Test for saponins

The stock solution from each crude extracts of (0.5 ml) was diluted with distilled water (20 ml) and then the test tube was shaken by hand for 15 min. The formation of a foam layer on the top of the test tube showed the presence of saponins.

Test for steroids

The powder samples of (1 g) were dissolved in chloroform (10 ml) and added concentrated sulphuric acid (1 ml) into the test tube by wall sides. The colour of the upper layer turned red and the sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Test for tannins

The stock crude extract solution (0.5 ml) was dissolved in chloroform (5 ml) and added acetic anhydride (1 ml). Finally sulphuric acid (1 ml) was added carefully to the solution along the wall sides of the vessel. A green colour was formed, showing the presence of tannins.

Test for triterpenoids

The dry crude plant extract (5g) was dissolved in chloroform (2 ml) and then acetic anhydride (1 ml) was added to it. One millilitre of concentrated sulphuric acid was added to the solution. The formation of reddish violet colour shows the presence of triterpenoids.

Antibacterial activity assay

The antibacterial potential test was carried out using the agar disc diffusion method. Negative controls were prepared by using the same solvents employed to dissolve the samples. Inhibition zones were measured and compared with the standard reference antibiotic amoxicillin. Each extract was subjected to serial dilution by using dimethyl sulphoxide (DMSO) as a solvent to give 2 mg/ml, 1 mg/ml, 0.5 mg/ml, and 0.25 mg/ml solutions. The concentration of amoxicillin standard used for this study was at 1 mg/ml. Each prepared concentration of the different extracts was tested for its antimicrobial activity against one gram (+) bacteria (*S. aureus*) and three gram (−) bacteria (*E. coli, K. pneumoniae* and *P. aeruginosa*) on nutrient agar plates using disc diffusion method. Whatman No. 1 sterile filter paper discs (6 mm diameter) were impregnated with methanol extracts or subfractions of *Khaya senegalensis* and placed on the inoculated agar. The concentration of amoxicillin standard used for this study was at 1 mg/ml. All the plates were incubated at 37 °C for 24 h. Evaluation of antibacterial activity was measured showing the diameter of the zones of inhibition against the tested bacteria. Each method in this experiment was replicated three times.

III. RESULTS

Table 1: Phytochemical analysis of methanolic and ethanolic leaves, barks and roots extract of *Khaya senegalensis*

Extracts	Phytochemicals					
	Alkaloids	**Flavanoids**	**Saponins**	**Steroids**	**Tannins**	**Triterpenoids**
Methanolic leaves extract	+++	++	+++	+	+++	-
Methanolic barks extract	++	++	+++	+	+	+
Methanolic roots extract	+++	++	+	++	+++	++
Ethanolic leaves extract	+++	++	++	+	++	-
Ethanolic barks extract	++	++	+	-	+	++
Ethanolic roots extract	+++	++	++	-	+	+

+++ = High; ++= Moderate; += Low ; − = Absence

Table 2: Antimicrobial activity of different crude extracts of *Khaya seenegalensis* against *E. coli, P.aeruginosa, K. pneumoniae* and *S. aureus*.

Crude Extract	Concentration	***E. coli***[a] (mm)		*S. aureus* (mm)		*P. aeruginosa* (mm)		*K. pneumonia* (mm)	
		Fresh leaves	Dry leaves	Fresh leaves	Dry leaves	Fresh leaves	Dry leaves	Fresh leaves	Dry leaves
Methanolic leaves extract	2 mg/ml	13± 0.14	10 ± 0.30	12 ± 0.20	8 ± 0.33	16 ± 0.22	6 ± 0.17		7 ± 0.29
	1 mg/ml	15 ± 0.23	10 ± 0.44	12 ± 0.41	11±0.14	13 ± 0.51	8 ± 0.41	7 ± 0.32	7 ± 0.27
	0.5 mg/ml	9 ± 0.18	8 ± 0.35	nd	12±.32	12 ± 0.27	6 ± 0.28	8 ± 0.18	8 ± 0.10
	0.25 mg/ml	8 ± 0.44	8 ± 0.28	10 ± 0.31	7 ± 0.32	6 ± 0.24		7 ± 0.29	nd
	Standard	30 ± 0.22	30 ± 0.10	26 ± 0.13	26 ± 0.34	7 ± 0.54	7 ± 0.23	8 ± 0.41	8 ± 0.28
Methanolic barks extract	1 mg/ml	11 ± 0.08	11 ± 0.30	11 ± 0.16	10 ± 0.21	13 ± 0.22	8 ± 0.34	7 ± 0.54	7 ± 0.17
	0.5 mg/ml	9 ± 0.23	7 ± 0.25	7 ± 0.15	8 ± 0.31	12 ± 0.41	6 ± 0.24	Nd	nd
	0.25 mg/ml	8 ± 0.12	6 ± 0.21	nd	nd	7 ± 0.12	7 ± 0.55	9 ± 0.20	9 ± 0.32
	Standard	30 ± 0.11	30 ± 0.23	20 ± 0.52	20 ± 0.22	7 ± 0.41	7 ± 0.56	7 ± 0.29	7 ± 0.08
	2 mg/ml	Nd	Nd	9±0.51	nd	10 ± 0.52	8 ± 0.21	8 ± 0.09	8 ± 0.09
Methanolic roots extract	1 mg/ml	13 ± 0.41	11 ± 0.25	12 ± 0.37	16 ± 0.32	8 ± 0.41	6 ± 0.22	7 ± 0.22	7 ± 0.22
	0.5 mg/ml	9 ± 0.23	8 ± 0.27	nd	nd	6 ± 0.41	6 ± 0.41	7 ± 0.12	7 ± 0.12
	0.25 mg/ml	8 ± 0.30	8 ± 0.37	11 ± 0.20	8 ± 0.26	nd	nd	7 ± 0.45	7 ± 0.14
	Standard	30 ± 0.31	30 ± 0.25	8 ± 0.45	8 ± 0.23	8 ± 0.41	8 ± 0.59	8 ± 0.05	8 ± 0.15
	2 mg/ml	17 ± 0.22	7 ± 0.23	12 ± 0.33	6 ± 0.34	10 ± 0.61	6 ± 0.21	7 ± 0.17	7 ± 0.29
Ethanolic leaves extract	1 mg/ml	12 ± 0.17	7 ± 0.28	9 ± 0.12	7 ± 0.34	8 ± 0.29	7 ± 0.49	8 ± 0.23	8 ± 0.54
	0.5 mg/ml	9 ± 0.20	nd	9 ± 0.09	nd	8 ± 0.37	8 ± 0.18	6 ± 0.28	nd
	0.25 mg/ml	9 ± 0.55	9 ± 0.39	8 ± 0.22	8 ± 0.12	7 ± 0.49	7 ± 0.23	Nd	nd
	Standard	10 ± 0.22	10 ± 0.37	7 ± 0.61	7 ± 0.27	8 ± 0.12	8 ± 0.34	9 ± 0.11	9 ± 0.19
	2 mg/ml	12 ± 0.43	6 ± 0.33	16 ± 0.20	12 ± 0.21	14 ± 0.09	6 ± 0.10	6 ± 0.22	6 ± 0.10
Ethanolic barks extract	2 mg/ml	16 ± 0.38	8 ± 0.12	16 ± 0.37	6 ± 0.44	17 ± 0.08	8 ± 0.17	6 ± 0.15	6 ± 0.39
	1 mg/ml	12 ± 0.19	6 ± 0.44	12 ± 0.55	6 ± 0.31	14 ± 0.12	8 ± 0.23	6 ± 0.28	6 ± 0.43
	0.5 mg/ml	13 ± 0.26	7 ± 0.33	10 ± 0.13	nd	8 ± 0.71	8 ± 0.12	7 ± 0.03	7 ± 0.33
	0.25 mg/ml	8 ± 0.13	8 ± 0.56	8 ± 0.22	8 ± 0.34	7 ± 0.12	7 ± 0.42	8 ± 0.61	8 ± 0.10
	Standard	10 ± 0.22	10 ± 0.24	7 ± 0.33	nd	11 ± 0.09	11 ± 0.32	7 ± 0.13	7 ± 0.32
Ethanolic le roots extract	2 mg/ml	10 ± 0.19	6 ± 0.44	12 ± 0.55	6 ± 0.31	14 ± 0.12	8 ± 0.23	6 ± 0.28	6 ± 0.43
	1 mg/ml	11 ± 0.20	9±0.23	10 ± 0.13	7 ± 0.33	8 ± 0.71	8 ± 0.12	7 ± 0.03	7 ± 0.33
	0.5 mg/ml	9± 0.11	8 ± 0.56	8 ± 0.22	8 ± 0.34	7 ± 0.12	7 ± 0.42	8 ± 0.61	8 ± 0.10
	0.25 mg/ml	8 ± 0.22	10 ± 0.24	7 ± 0.33	10±0.45	11 ± 0.09	11 ± 0.32	7 ± 0.13	7 ± 0.32
	Standard	10 ± 0.29	6 ± 0.44	12 ± 0.55	6 ± 0.31	14 ± 0.12	8 ± 0.23	6 ± 0.28	6 ± 0.43

nd = Not detected

IV. DISCUSSION

So many studies have reported the phytochemical constituents in the plant samples are known to be biologically active compounds and they are responsible for different activities such as antioxidant, antihelmentic, antimicrobial, antifungal, and anticancer (5, 7). All secondary metabolite components displayed antimicrobial properties through different biological mechanisms. Most of the secondary metabolite components were isolated and identified in the polar plant crude extracts (6, 8). The phytochemical screening of methanolic and ethanolic crude extracts from fresh and dry powder leaves, barks and roots samples of *Khaya senegalensis* used in this study revealed that the crude extracts contained alkaloids, flavonoids, saponins and tannins in all the extracts (Table 1). It also revealed the presence of steroids and triterpenoids but not in all the extracts (Table 1). The most effective bioactive compounds alkaloids and flavonoids were found in both methanolic and ethanolic crude extracts. Tannins are another active compound found to be present in all extracts. Therefore, the detected different *Khaya senegalensis* bioactive compounds in different crude extracts from dry and fresh leaves, barks and roots of *Khaya senegalensis* may be responsible for the antibacterial activities. Several reports are available on flavonoid groups which exhibited high potential biological activities such as antioxidant, anti-inflammatory, antimicrobial, anti-angionic, anticancer and anti-allergic reactions (4, 5). Saponins and tannins are also bioactive constituent which involved in plant defense system because of their antimicrobial activity. The antimicrobial activity of the fresh and dry plant crude extracts was estimated using standard conventional methods against *S. aureus*, *E. coli*, *P. aeruginosa* and *K. pneumoniae*. The dry methanolic crude extract of *Khaya senegalensis* and its fractions revealed comparatively small antibacterial potential against gram-positive and gram-negative bacteria at the concentrations of 2 mg/ml, 1 mg/ml, 0.5 mg/ml and 0.25 mg/ml with their respective zones of inhibition of 0–11 mm (Table 2). However, the fresh methanolic crude extract of *Khaya senegalensis* and its fractions revealed a moderate antibacterial potential against the employed bacterial strains and all working concentrations with their respective zones of inhibition of 0–17 mm (Table 2). The methanolic fresh crude extract showed moderate antibacterial potential against *S. aureus*, *E. coli* and *P. aeruginosa* bacteria, at the concentrations of 2 mg/ml, 1 mg/ml and 0.5 mg/ml (Table 2). However, all crude extracts from dry samples showed small activity against all employed bacterial strains. Ethanolic crude extract from fresh samples showed moderate activity against *S. aureus*, *E. coli*, *P. aeruginosa* and *K. pneumoniae* at concentrations 2 mg/ml and 1 mg/ml but dry crude extract samples showed small potential at most of the concentrations against all bacterial strains. However, the bacterium *K. pneumoniae* did not show any potential activity at the concentration of 0.5 mg/ml and 0.25 mg/ml. The ethanolic crude extracts from fresh and dry samples has shown any activity against *E. coli* and *S. aureus* at the concentration of 2 mg/ml. *P. aeruginosa* and *K. pneumoniae*. All subfractions showed moderate antibacterial potential against most of the tested bacteria. Generally, the antimicrobial activity of plant crude extracts depends on the dose and the type of bacterial strains employed (4). Also these antibacterial actions could be related to their chemical components in the crude extracts. The bioactive compounds such as tannins and flavonoids components were present in the crude extracts. However, these bioactive compounds were inducing antimicrobial activities (9). The amount of active components in the crude extract may be diluted or increased their concentrations by fractionation, because they have the ability to inactivate microbial activity, enzymes, cell envelope transport proteins, and so forth (5, 9). Further studies are required for the isolation and identification of individual active compounds and also *in vivo* studies are needed for better understanding of their mechanism of action as antimicrobials.

V. CONCLUSION

This study that focused on antimicrobial study of different crude extracts and showed that *Khaya senegalensis* methanolic crude extract from fresh leaves extract and from dry leave shows highest activity against the employed bacteria. Phytochemical screening showed that the antibacterial activities of the crude extracts of *Khaya senegalensis* depend on the presence of phytochemicals such as alkaloids, steroids, tripertenoids, flavonoids and tannins. This plant crude extracts could serve as potential sources of new antimicrobial agents. Further research is needed towards isolation and identification of active principles present in the extracts which could be used for pharmaceutical use.

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