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Antimicrobial and antioxidant activities of ethanolic stem bark and root extracts of *Khaya ivorensis* A Chev. (Meliaceae)

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Abstract
Plants of the genus *Khaya* (Meliaceae) are traditionally used to relieve ear infection, fever, malaria. The main objective was to evaluate the antimicrobial and the antioxidant activity of the ethanolic of extracts stem back and root of *Khaya ivorensis*. Plants were extracted by ethanol. The antimicrobial activity was assayed against some Gram-negative bacteria (*Klebsiella pneumonia* ATCC 70603, *Haemophilus influenza* ATCC 49247), Gram-positive bacteria (*Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* BAA-917, *Enterococcus faecalis* ATCC 51299) and fungal (*Candida albicans* ATCC NR-2450,*Candida albicans* 141S, *Candida albicans* NR- 2450, *Candida glabrata* 44B, *Candida parapsilosis* 153B, *Candida krusei*) by determining the Minimum Inhibitory Concentration (MIC) and the antioxidant activity by determining the inhibition concentration IC_{50} using DPPH, FRAP and ABTS methods. The phytochemical screening of *K. Ivorensis* showed the presence of alkaloids, flavonoids, tannins, saponins, steroids and triterpenoids. The ethanolic extracts of stem back and root showed significant activity against two Gram-positive strains (*S. aureus* BAA-917 and *S. areus* ATCC 43300) with MIC ranging 0.3125 to 0.625 mg/mL and two fungal strains (*C. albicans* 141S and *C. krusei*) with MIC 0.625 mg/mL. The extract had the greatest scavenging activity with IC_{50} ranging 2.08, 4.48 µg/mL and 2.78 µg/mL in ABTS and DPPH assays respectively. These results suggest that extracts from *K. Ivorensis* possess antimicrobial and antioxidant activities and therefore justifies their usage in traditional medicine for the treatment of various diseases.

Keywords: *Khaya ivorensis*, phytochemical screening, antioxidant, antimicrobial

Introduction
Although, a number of antibiotics are widely used in medicine, the use of plants as antimicrobials agent is gradually attracting probably due the high cost, unavailability and resistance of drugs. The medicinal flora in the tropical region has a preponderance of plants that provide raw materials for addressing a range of medical disorders and pharmaceutical requirements (Fatope *et al*., 2001) [10]. *Khaya ivorensis* A CHEV (Meliaceae) commonly known as African mahogany, is a large tree widely diffused in Africa from the Guinea coast to Cameroon and extending eastward through Congo basin to Uganda and some part of Sudan (Nakatani *et al*., 2001) [19]. In Cameroon and in most developing countries, the decoction of extracts of *Khaya ivorensis* has a history of use in traditional medicine for the treatment but also for the prevention of several diseases. Traditional preparations include concoctions, decoctions, infusions or macerations using different plant parts from one of several species. Its bitter bark is mostly the part that is used to make decoction to treat some illness like fever, rheumatism, gastric pains, cough and remedy against worm infection (Lemmens, 2008) [16]. This plant has been demonstrated to exhibit a wide range of biological properties, such as anti-inflammatory (Agbedahunsi *et al*., 2004) [3], anti-malarial (Nzangue *et al*., 2011) [20], anti-fungal (Abdelgaleil *et al*., 2005) [2] and antifeeding properties (Vanucci *et al*., 1992) [27]. Various classes of the chemical constituents were isolated from stem bark, leaves and roots from *khaya* (Zhang *et al*., 2009) [28] and major compounds have been attributed to a limonoids. The objective of this study, was evaluate *in vitro* antimicrobial and the antioxidant activities of the ethanolic extracts of stem back and root from *Khaya ivorensis*.

Materials and Method
Collection and identification of plant materials
Khaya ivorensis stem bark and root material was collected in April 2019 in Mont Kala, Bankomo Sub-division, and Centre Region and identified by Mr. Victor NANA, a plant taxonomist. Voucher Specimens are deposited under N° 52660 at the National Herbarium of Cameroon (Yaounde).

Preparation of plant extracts

The air-dried stem barks (3.5 kg) and root (5.5 kg) of *K. Ivorensis* were exhaustively macerated with 11 L and 15 L of ethanol at room temperature through percolation for 72h respectively. The macerates were filtered with What man no.1 filter paper, the solvents were evaporated under reduced pressure in a rotary evaporator at 40 ºC to afford dark crude extract (SBKI, 385 g) and (RKI, 895 g) respectively.

Phytochemical screening of plant extracts

The extracts were subjected to phytochemical screening to detect the presence of alkaloids, flavonoids, tannins, saponins, steroids and triterpenoids. The method of Harborne (1992) [11]. And Trease and Evans (1989) [26] were employed.

Antimicrobial Assay

Microbial strains

Five bacteria strains were used as test microorganisms in this study. One Gram-Positve bacteria, *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* BAA-917, *Enterococcus faecalis* ATCC 51299 and Gram-negative, Klebsiella *pneumonia* ATCC 70603, *Haemophilus influenzae* ATCC 49247, were obtained from Galenic Pharmaceutical Laboratory Microbiology section of the Departement of Galenic Pharmacy and Legislation of the Faculty of Medecine and Biomedical Sciences of the University of Yaounde1. *Candida albicans* ATCC NR-2450 reference strain provided by BEI Resources NIAID, NIH, and *Candida albicans* 141S, *Candida albicans* NR- 2450, *Candida glabrata* 44B, *Candida parapsilosis* 153B, *Candida krusei* isolates obtained from HIV positive patients presenting at the Yaoundé Central Hospital (Cameroon), and developing various candidiasis (vaginal, oro-pharyngeal, intestinal, urinary).

Inoculum preparation

Before any test, bacteria strains were subcultured on Mueller Hinton agar slants at 35°C for 18h. Mature colonies were collected with inoculating loop and introduced into a tube with 5mL of sterile saline (0.9 % NaCl) and homogenized. The turbidity of the solution was adjusted at 0.5 McFarland standards. Inoculum of each yeast isolate and strains was prepared from a 2 days old culture on Sabouraud Dextrose Broth (SDB) at 37 °C. The suspension was adjusted to 2.5x 10^3 cells/mL using sterile 0.9% NaCl and Malassez counting chamber under an optical microscope (Human Scope Light, Human).

Antimicrobial assays

The broth micro-dilution method was used to assess yeasts and bacteria susceptibility to extracts and natural products. The CLSI M27-A3 (CLSI, 2008) and CLSI M7-A10 (CLSI, 2009) methodologies were used for yeast and bacteria respectively. Briefly, 100 µL of each extract or natural product were dissolved respectively at 10 mg/mL and 1 mg/mL in 1 % DMSO and serially diluted using SDB in 96-well plates. Positive control consisted of Fluconazol (Forcan-200, Cipla Pharmaceuticals, India) at 10 mg/mL. 100 µL of standardized inoculum were added into each well for a final volume of 200 µL. The final tested plant product concentrations ranged from 10 to 0,156 mg/mL for the crude extracts, 1 to 0, 0156 mg/mL for Fluconazol. Negative control wells consisted of inoculum with equivalent volume of 1% DMSO and no drugs added. The lowest concentration with no visual change in turbidity (indicating no growth of microorganism) was considered as the Minimum Inhibitory Concentration (MIC) at 37 °C, after 24 h or 48 h of incubation for bacteria and yeasts respectively. The Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) were determined by subculturing 25 µL aliquots of the clear wells into 100 µL of freshly prepared broth medium and incubating at 37 °C for 24 h or 48 h for bacteria and yeasts respectively. The lowest concentration of test sample showing no turbidity change was considered as MFC or MBC. All tests were performed in triplicate. Wells without inoculum or extract were included in each plate to control the background sterility and growth. The type of bacterial or antifungal effect of extract or fraction was deduced from the calculated MFC/MIC, MBC/MIC ratio, and identified as fungicidal or bactericidal when MFC/MIC, MBC/MIC $\leq$ 4, or fungistatic, bacteristatic when MFC/MIC, MBC/MIC > 4 (Carbonnelle *et al*., 1987) [8].

Antioxidant Activity

ABTS radical activity

The ABTS radical scavenging capacity of the samples was measured with modification to a 96-well microtitre plate format as described by Re *et al*. (1999) [23]. With slight modifications. ABTS radical was generated by reacting 7 mM solution of ABTS and 2.45 mM solution of potassium per sulfate at room temperature for 12 h. The ABTS radical stock solution was adjusted to 7.00 ± 0.02 at 734 nm before use. The test samples (40 µL) were made in a concentration range of 0.78 to 100 µg /mL by two fold serial dilutions and 160 µL of ABTS radical solution was added. Absorbance was measured after 6 min at 734 nm. Trolox and ascorbic acid were used as positive controls, methanol as negative control and compound without ABTS as blank. Percentage of ABTS•+ inhibition was calculated using formula:

Scavenging capacity (%) = 100 - [(absorbance of sample - absorbance of sample blank) × 100/ (absorbance of control) - (absorbance of control blank)]. The IC_{50} values were estimated from the percent inhibition versus concentration plot derived from the percentage scavenging activity.

DPPH assay

The DPPH radical-scavenging activity was determined using the method proposed by Brand-Williams *et al*. (1995) [7]. with some modifications to 96-well microtitre plate. Various concentrations of compounds in methanol were prepared (7.81 to 1000 µg /mL). Ascorbic acid and Trolox were used as a positive control at concentration of 100 to 0.78 µg/mL. Then, 160 µL of DPPH (0.037 mg/mL) in methanol was added to 40 µL of the test solution, or standard, and allowed to stand at room temperature in a dark for 30 min. Methanol was used as a blank. The change in colour from deep violet to light yellow was then measured at 517 nm using a Versa ax micro plate reader. Results were expressed as percentage reduction of the initial DPPH absorption in relation to the control. The concentration of compound that reduced DPPH colour by 50 % (IC_{50}) was determined as for ABTS•+.

Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP assay was carried out according to the procedure of Benzie and Strain (1996) [6]. With slight modifications. Briefly, the FRAP reagent was prepared from acetate buffer (pH 3.6), 10 mmol 2,4,6-tripyridyl-S-triazine (TPTZ) solution in 40 mmol HCl and 20 mmol iron (III) chloride solution in proportions of 10:1: 1 (v/v), respectively. The FRAP reagent was freshly prepared and was warmed to 37 º C in a water bath prior to use. Fifty microliters of sample were added to

1.5 mL of the FRAP reagent. The absorbance of the reaction mixture was then recorded at 593 nm after 4 min. The standard curve was constructed using $FeSO_4$ solution (0.1-2 mM), and the results were expressed as µmol $FeSO_4$/g dry weight of compound. All the measurements were taken in triplicate and the mean values were calculated.

Results and Discussion

Phytochemical screening

The phytochemical investigations of the stem bark and root of *K. Ivorensis* revealed the presence of flavonoids, triterpenoids, tannins, phenols, alkaloids, saponins and glycosides (Table 1). This result can be explained by the fact that during maceration, secondary metabolites are separated according to their affinity and solubility with extraction solvent (Cowan MM, 1999) [9]. These secondary metabolites were previously found in some extracts of these plants' species (Zhang *et al*., 2011) [28]

Table 1: Phytochemical analysis of ethanolic sterm bark and root extract of *Khaya ivorensis.*

Phytochemical component	SBKI	RKI
Flavonoids	+++	+++
Triterpenoids	+++	+++
Tannins	+++	+++
Phenols	+++	+++
Alkaloids	+ +	+ +
Saponins	+ +	-
Glycosides	+ +	-

SBKI: Ethanolic extract of *Khaya ivorensis* stem bark; RKI: Ethanolic extract of *Khaya ivorensis* root. +++ = High; ++= Moderate; += Low; − = Absent.

Antimicrobial activity

The results of the MIC, MBC, MFC, MIC/MBC and MFC/MIC are represented in Tables 2 and 3 below. The antibacterial and anti candidal effect of extracts was evaluated by calculating the ratio of MBC/MIC and MFC/MIC. If MBC/MIC or MFC/MIC was ≤ 4, the extracts was defined as bactericidal or fungicidal respectively, where as if MBC/MIC or MFC/MIC was > 4, the extract was defined as bacteriostatic or fungistatic (Carbonnelle *et al*., 1987) [8].

The minimum inhibitory concentration ranged from 0.3125±0.00 to 2.5±0.00 mg/mL on all the tested microorganisms. Amongst the crude extract, the best activities were observed with the SBKI crude extract on *S. aureus* BAA-917 (0.3125±0.00 mg/mL). All crude extract showed good activity on the all tested candida (Table 3). This activity of crude extract is less than chloramphenicol and fluconazol using as positive control. The plant chosen for this study are commonly used for treating infectious diseases in herbal therapy and are known to produce a wide range of bioactive compounds; including antimicrobials (Idu *et al*., 2010) [13]. Following the classification of Carbonnnelle *et al*., ethanolic extracts (SBKI and RKI) showed significant activity against all tested bacteria except *H. influenza* ATCC 49247 with MIC ranging 0.3125±0.00 to 2.5±0.00 mg/mL. Therefore, the bactericidal activity was observed against *E. faecolis* ATCC 51219 with MBC/MIC = 4 mg/mL and fungicidal against all tested candida (Tables 2 and 3). The observed antimicrobial activity was certainly due to the presence of various classes of secondary metabolites within the crude extracts such as tannins, saponins, steroids, flavonoids and triterpenoids. These metabolites may exert their inhibitory effect through different mechanisms. In fact, it is known that tannins exert their antimicrobial activity by binding with proteins and adhesins, inhibiting enzymes, complexation with the cell wall and metal ions, or disruption of the plasma membrane (Cowan MM, 1999) [9]. On the other hand, saponins have the ability to cause leakage of proteins and certain enzymes from the cell (Marjorie MC, 1999) [17]. The sensitivity of steroids and the membrane lipids indicate their specific association that causes leakage from liposomes (Moon and Shibamoto, 2009) [18]. Flavonoids have the ability to complex with proteins and bacterial cells forming irreversible complexes mainly with nucleophile amino acids. This complex often leads to inactivation of the protein and loss of its function (Shimada *et al*., 2006) [24]. Hence, the presence of these compounds in *K. Ivorensis* may explain the antibacterial activities observed. However, further studies are needed to isolate and characterize the active ingredients responsible for the efficacy of the most active extracts. Triterpenoids isolated from various parts of plants have been reported to have anti-inflammatory activity (Ismaili *et al*., 2002) [15] bactericidal analgesic, antiviral and antiallergic (Patocka *et al*., 2003) [21]. The presence of these phytochemicals in stem bark and root suggest that the plant is pharmacologically active and supporting the claim by traditional healers.

Antioxidant activity

In this study, the antioxidant activity of extracts was determined using the free radical 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the ferric reducing antioxidant power (FRAP) assays. The antioxidant activity of extracts can be determined *in vitro* by hydrogen atom transfer (HAT) method and single electron transfer (SET) method. HAT methods measure the capacity of an antioxidant to scavenge free radical by hydrogen donation to form a stable compound. SET methods determine the ability of the antioxidant to transfer one electron to reduce compound including metals, carbonyls and radicals (Huang *et al*., 2005) [12]. FRAP assay involves SET method, while DPPH and ABTS assay involve both method predominantly via SET method (Becker *et al*., 2014) [5]. From the dose-response activities, the IC_{50} values were obtained and presented in Table 4. The IC_{50} values for the different extracts ranged from 2.08 µg/mL to 4.48 µg/mL in DPPH assay, from 2.78 µg/mL to 16.35 µg/mL in ABTS assay and from 7.39 µg/mL to 10.98µg/mL in FRAP assay (Table 4).

Table 2: Antibacterial activity of ethanolic crude extracts of *Khaya ivorensis*

Test samples		MIC and MBC ± SD (mg/mL)														
		***S. areus* ATCC 43300**			***S. aureus* BAA-917**			***K. pneumonia* ATCC 70603**			***E. faecalis* ATCC 51219**			***H. influenza* ATCC 49247**		
		MIC	**MBC**	**MBC/MIC**	**MIC**	**MBC**	**MBC/MIC**	**MIC**	**MBC**	**MBC/MIC**	**MIC**	**MBC**	**MBC/MIC**	**MIC**	**MBC**	**MBC/MIC**
Crude extracts	SBKI	0.625±0.00	> 10	ND	0.3125±0.00	5	16	2.5±0.00	2.5±0.00	ND	1.25±0.00	5	4	1.25±0.00	> 10	ND
	RKI	0.625±0.00	> 10	ND	0.625±0.00	5	8	1.25±0.00	5±0.00	ND	1.25±0.00	5	4	> 10	ND	ND
Reference compound	Chlo	0.0625±0.00	0.0625±0.00	1	1.00±0.00	> 1	ND	0.5±0.00	> 1	ND	0.0625±0.00	0.0625±0.00	1	1±0.00	> 1	ND

S. areus ATCC 43300: *Staphylococcus aureus* ATCC 43300; *S. areus* BAA-917: *Staphylococcus aureus* BAA-917; *K. pneumonia* ATCC 70603: *Klebsiella pneumonia* ATCC 70603; *E. faecalis* ATCC 51219: *Enterococcus faecalis* ATCC 51299; *H. influenza* ATCC 49247: *Haemophilus influenzae* ATCC 49247; MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; SD: Standard Deviation; ND: not determined.;SBKI: Ethanolic extract of *Khaya ivorensis* bark ; RKI: Ethanolic extract of *Khaya ivorensis* root; Chlo: chloramphenicol.

Table 3: Antifungal activity of ethanolic crude extracts of *Khaya ivorensis*

Test samples		MIC and MFC ± SD (mg/mL)														
		***C. albicans* NR-2450**			***C. albicans* 141S**			***C. glabrata* 44B**			***C. krusei***			***C. parapsilosis* 153B**		
		MIC	**MFC**	**MFC/MIC**	**MIC**	**MFC**	**MFC/MIC**	**MIC**	**MFC**	**MFC/MIC**	**MIC**	**MFC**	**MFC/MIC**	**MIC**	**MFC**	**MFC/MIC**
Crude extracts	SBKI	1.25±0.00	5±0.00	4	0.625±0.00	1.25±0.00	2	1.25±0.00	5±0.00	4	1.25±0.00	2.5±0.00	2	1.25±0.00	5±0.00	4
	RKI	1.25±0.00	5±0.00	4	0.625±0.00	1.25±0.00	2	1.25±0.00	5±0.00	4	0.625±0.00	0.625±0.00	1	1.25±0.00	5±0.00	4
Reference compound	Fluc	0.0156±0.00	0.0625±.00	4	0.0625±0.00	>1	ND	0.125±0.00	0.125±0.00	1	0.015625±0.00	0.0312±0.00	2	0.015625±0.00	0.0312±0.00	2

C. albicans NR-2450: *Candida albicans* ATCC NR-2450 *C. albicans* 141S: *Candida albicans* 141S; *C. glabrata* 44B: *Candida glabrata* 44B; *C. krusei Candida krusei*; *C. parapsilosis* 153B: *Candida parapsilosis* 153B; MIC: Minimum Inhibitory Concentration; MFC: Minimum Fungicidal Concentration; SD: Standard Deviation; ND: not determined; SBKI: Ethanolic extract of *Khaya ivorensis* bark; RKI: Ethanolic extract of *Khaya ivorensis* root; Fluc: flucazol.

Table 4: Antioxidant activity of ethanolic crude extracts of stem back and root of *Khaya ivorensis*

Extracts	Dpph IC_{50} (µG/ML)	Abts IC_{50} (µG/ML)	Frap IC_{50} (µG/ML)
SBKI	4.48±0.02	2.78±0.49	10.98±13.70
RKI	2.08±0.19	16.35±0.29	7.34±1.06
Trolox	5.36±0.10	3.71±0.21	Nd
Ascorbic acid	2.80±0.03	2.61±0.08	Nd

The IC_{50} values of SBKI and RKI extracts were significantly different from the IC_{50} of ascorbic acid and Trolox, standard antioxidant agents used as a positive control. The capacity of flavonoids to act as antioxidants *in vitro* has been previously studied (Pietta PG, 2000) [22]. RKI extract exhibited the highest DPPH inhibitory activity among the extract while SBKI was potent in scavenging the DPPH radicals. Almost similar results were obtained with ABTS *radical*. The scavenging of the ABTS+ radical by the extracts was found to be much higher than that of DPPH radical. Many factors such as the stereoselctivity of the radical, the solubility of the extract in different testing systems have been reported to affect the capacity of extracts to react and quench different radicals. The IC_{50} values in the FRAP assay of the different extracts of the roots and stem bark of *K. Ivorensis* were significantly lower than that of ascorbic acid and Trolox.

Conclusion

The results of this study provided an insight into the antimicrobial and antioxidant properties of
the extracts of *K. Ivorensis* used traditionally for the prevention and treatment of various infectious and noninfectious problems, as well as opportunity for selection of bioactive extracts for initial fractionation and isolation of natural bioactive compounds.

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