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Antimicrobial, free radical scavenging and cytotoxic activities of *Khaya grandifoliola* C.DC extracts

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ABSTRACT

Aims: *Khaya grandifoliola* C.DC is a plant used locally in Nigeria ethno medicine for remedy of various disease conditions. However, there is little scientific evidence to support the therapeutic claims of the plant. Therefore, these investigations were conducted to determine the antimicrobial activity, antioxidant and cytotoxic potentials of the plant extracts.
Methodology and results: *In vitro* antimicrobial activity of the leaf and stem bark extracts of *K. grandifoliola* against some human pathogens was done using agar diffusion method. The free radical scavenging activity and cytotoxic property of the plant materials were evaluated using 2, 2- diphenyl-1-pieryhydrazyl radical (DPPH) and brine shrimp lethality bioassay methods respectively. The yields of the plant material extracts ranged from 3.57±0.06 to 6.49±0.01% and 4.76±0.02 to 9.17±0.06% for the leaf and stem bark extracts respectively. The minimum inhibitory concentration (MIC) of KG-A and KG-E ranged from 2.5 to 200 mg/mL and recorded remarkable activity against the growth of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Trichophyton rubrum* and *Aspergillus flavus*. However, *Strepcoccus pneumoniae*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, and *T. rubrum* were resistant to the KG-W. The plant extracts demonstrated high DPPH free radical scavenging activity when compared with ascorbic acid used as control in the assay and, also exhibited lethality against brine shrimp larvae with LC_{50} values ranging from: leaf extracts (0.67 to 1502 ppm) and stem bark extracts (0.91 to 1431 ppm).
Conclusion, significance and impact of study: The results show that the KG-A and KG-E have great potentials as antimicrobial agent and may be used in the treatment of infectious diseases caused by the susceptible organisms.

Keywords: Antimicrobial, *Khaya garandifoliola* C.DC, MIC, lethality bioassay

INTRODUCTION

Medicinal plants have been used to treat various human diseases and recently, there has been wide spread interest in botanical medicines (Nagreeb *et al.*, 2013). According to the world health organization (WHO) more than 80% of the world population relies on traditional medicine for their primary health care needs (Vashit and Jundal, 2012). There have been massive research interests in essential oils and extracts from plant origin as potential antimicrobial, antioxidant and anticancer agents. There is also increasing interest in the radical scavenging activities of some natural antioxidants, especially those found in medicinal plant, which may play a role in preventing various chronic diseases (Dhekra *et al*., 2014; Gregoria, 2015).

Khaya grandifoliola (also called African mahogany) is a species of plant in the Meliaceae family. It grows up to 40 m high and 5 m in girth. The bark is usually grey and brings out bitter exudates which are gummy in nature when broken. Odugbemi *et al.* (2007) and Olowokudejo *et al.* (2008) in their respective studies reported that the plant is used in the form of concoction for the treatment of convulsion, cough, stomach ache, fever, threatened abortion, rheumatism, dermatomycosis and malaria in Nigeria.

Based on the ethnomedical information on the plant, the present investigation was aimed at demonstrating the antimicrobial, antioxidant and cytotoxic activities of *K. grandifoliola* leaf and stem bark extracts.

MATERIALS AND METHODS

Collection, identification and extraction method

Fresh leaf and stem bark of *K. grandifoliola* were harvested from uncultivated farmland located in Owo, Ondo State, Nigeria in May, 2011. The plant materials were then authenticated at the Herbarium Section of the Department of Botany, University of Lagos. Voucher specimen (LVH3616) was deposited at the Department of Forestry and Wood Technology, Federal University of

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Technology, Akure. The authenticated plant materials were washed and cleaned thoroughly with tap water and then air-dried under shade. The dried samples were then ground into coarse powder with the aid of a mechanical grinder and, stored in clean air- tight containers, and kept in a cool, dry place until required for use.

A 100 g portion of the respective powdered sample was soaked separately and concurrently in 300 mL of different solvents (acetone, ethanol and water) for 72 h with intermittent stirring using sterile spatula. The plant extracts were then filtered through Whatman No1. filter paper and kept in bijou bottles after drying using rotary evaporator at a temperature of 50 °C to yield crude extracts (Sneh *et al.*, 2014) that were labeled KG-ELE, KG-ALE, KG-WLE, KG-ESE, KG-ASE and KG-WSE respectively. Different concentrations of the respective extracts were prepared by diluting 0.10 g, 0.20 g, 0.30 g, 0.40 g and 0.50 g of the extracts in 100 mL of 0.01% Tween-20 to obtain concentrations of 10 mg/mL, 20 mg/mL, 30 mg/mL, 40 mg/mL and 50 mg/mL respectively (Idu and Igeleke, 2012).

Test microorganisms

The microorganisms employed in this study were fifteen clinical isolates (*Bacillus subtilis*, *E. coli*, *Enterococcus faecalis*, *K. pneumonia*, *P. aeruginosa*, *S. typhi*, *S. aureus*, *S. epidermidis*, *Streptococcus* spp., *Streptococcus pyogenes*, *A. flavus*, *Candida albicans*, *C. glabrata*, *Cryptococcus neoformans* and *T. rubrum*) and five typed cultures (*B. substilis* ATCC 6633, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *S. typhi* ATCC 6539 and *C. albicans* ATCC 10231) obtained from Federal Medical Center, Owo and Federal Institute of Industrial Research, Oshodi, Lagos State, Nigeria respectively.

***In vitro* antimicrobial susceptibility test**

The extracts obtained from the test plant materials were screened against the test organisms by agar well diffusion method (Perez *et al.*, 1990). A 25 mL aliquot of Mueller-Hinton agar (MHA, Lab Oratorios Britania, Argentinia) was poured into each Petri plate. When the agar solidified, test organisms were inoculated on the surface of the plates (1×10^6 CFU/mL and 1×10^6 SFU/mL for bacteria and fungi respectively) using a sterile glass spreader, allowed to set and punched with 6mm cork borer. A portion of 50 µL of each of the extract concentrations was introduced into the wells. Wells containing the same volume of 30% Dimethyl sulphoside (DMSO) served as negative control, while chloramphenicol (100 µL) and miconazole (100 µL) were used as positive controls for bacterial and fungal plates respectively. The tests were carried out in triplicates. Bacterial plates were incubated at 37 °C while fungal plates were incubated at 25 °C for 24 h and 72 h respectively. The diameters of the zones of inhibition were then measured in millimeters.

Minimum inhibitory concentration (MIC)

Twofold serial dilutions of the extracts were prepared in Mueller-Hilton broth and Saboraud broth for bacteria and fungi respectively to achieve a decreasing concentrations ranging from the least concentration that produced clear zone of inhibition (10 mg/mL to 0.156 mg/mL). All tubes including the controls were labeled accordingly. Each dilution was seeded with 1 mL of standardized inoculums (1.0×10^6 CFU/ml for bacteria and 1.0×10^6 SFU/ml for fungi) incubated at 37 °C for 24 h and 25 °C for 72 h for bacteria and fungi respectively. A tube containing only seeded broth (i.e. without plant extracts) was used as the positive control while the un-inoculated tube was used as negative control. The lowest concentration of each extract that showed a clear zone of inhibition was when compared with the controls was considered as the MIC.

DPPH free radicals scavenging assay

The DPPH free radical (1,1-diphenyl-2- picrylhydrazyl) scavenging assay was determined using the method described by Ngbolua *et al.* (2014) and Koto-te-nyiwa *et al.* (2014). A 4 mg portion of DPPH was dissolved in methanol to obtain 100 µM methanol solution of DPPH. A 3 mL portion of the extract concentrations (0.00 to 2.0 mg/mL) was added to 1 mL of 100 µM methanol solution of DPPH. The mixture was shaken vigorously and incubated in the dark at room temperature for 30 min. The absorbance at 517 nm was measured against the blank (methanol) and ascorbic acid as positive control using a spectrophotometer. The DPPH radical scavenging activity (%) was then determined by the following equation:

$$\text{DPPH radical scavenging: Activity (\%)} = [(Ao - As) / Ao] \times 100$$

where Ao =absorbance of DPPH without sample; As = absorbance of mixture of sample and DPPH. The radical scavenging activity of the samples (Median inhibitory concentration, IC_{50}) value was determined from an equation line obtained by plotting a graph of concentration against percentage inhibition.

Determination of cytotoxic effect of plant extracts

The brine shrimp (*Artemia salina*) lethality bioassay was carried out according to the method described by Haq *et al.* (2012). Brine shrimp eggs were hatched in artificial sea water prepared by dissolving 38 g of salt in 1 L of distilled water, filtered and put in shallow rectangular dish. A plastic divider with several holes of 2 mm size was clamped in the dish to make 2 equal compartments. Brine shrimp eggs were placed in one side of the compartment while the other compartment was illuminated. After 48 h of illumination, phototrophic nauplii (Brine shrimp larvae) were collected by using pipette from the lightened side. Samples were then prepared by dissolving 20 mg each of the extracts in 2 mL of DMSO from where further diluted concentrations of 1000, 100, 10 and 1 ppm were

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prepared. A 4 mL portion of the artificial sea water was added into each test tube and 20 shrimps were transferred into it. This was followed by the addition of 1 mL of each of the test extracts of previously prepared concentrations and maintained under illumination at room temperature. Survivors were counted with the aid of magnifying glass after 24 h. The percentage mortality was calculated using Abbot's formula and the LC_{50} was also determined (Meyer *et al.*, 1982; Abbot, 1987).

Data analysis

Data were presented as mean±standard error (SE). Significance difference between different groups was tested using two-way analysis of variance (ANOVA) and treatment means were compared with Duncan's New Multiple Range Test (DNMRT) using SSPS window 7 version17.0 software. The significance was determined at the level of $p \leq 0.05$.

RESULTS AND DISCUSSION

In the present investigation, the inhibitory effect of different *K. grandifoliola* extracts (KG-ELE, KG-ALE, KG-WLE, KG-ESE, KG-ASE and KG-WSE) were evaluated for their antimicrobial, antioxidant and cytotoxic activities. The average yields of the extracts of the plant materials varied between KG-WLE (3.57±0.06) and KG-ASE (9.17±0.06) as presented in Table 1. The stem back exhibited better yields than the leaf extracts in most of the solvents. The differences in the yields obtained from the three solvents could be attributed to the polarity of the individual solvent and the types of phytochemicals present. Acetone was considered to be the best solvent, followed by ethanol for the extraction of the plant materials. These findings agreed with earlier investigations by Koto-te-nyiwa *et al.* (2014) and Koffi *et al.* (2015) who worked on similar medicinal plants. In their respective studies, they attributed the high and low yields experienced in the plant parts to their respective solubilities and antioxidant capacities.

Table1: The percentage yields of *K. grandifoliola* extracts.

Plant extract	% yield
KG-ELE	5.86±0.03
KG-ALE	6.49±0.01
KG-WLE	3.57±0.06
KG-ESE	4.76±0.02
KG-ASE	9.17±0.06
KG-WSE	5.62±0.03

Key: KG-ELE, *K. grandifoiliola* ethanol leaf extract; KG-ALE, *K. grandifoiliola* acetone leaf extract; KGWLE, *K. grandifoiliola* water leaf extract; KG-ESE, *K. grandifoiliola* ethanol stem bark extract; KG-ASE, *K. grandifoiliola* acetone stem bark extract; KG-WSE, *K. grandifoiliola* water stem bark extract.

The DPPH assay has been widely used to analyze the antioxidant activity of plant extracts and foods as free radical scavengers or hydrogen donors (Soares *et al.*, 1997). The antiradical scavenging activity of the different extracts of *K. grandifoliola* may be related to the nature of the phytochemical constituents of the plant materials.

The percentage inhibition of the extracts increased as the concentration of the extracts increased. This phenomenon agrees with the report of Abeer *et al.* (2013) on antioxidant properties of some plant extracts. The LC_{50} demonstrated a range of 1.27±0.00 to 1.69±0.10 and 1.40±0.03 to 1.71±0.00 for the leaf and stem bark extracts of the plant respectively.

The values of LC_{50} obtained were significant ($p \leq 0.05$) higher than 0.40±0.15 obtained for ascorbic acid used as standard (Table 2). Since lower IC_{50} value corresponds to higher antioxidant activity (Vinod *et al.*, 2014). It is evident that KG-ELE, KG-ALE, KG-ESE and KG-ASE demonstrated higher antioxidant activity than KG-WLE and KG-WSE. The high apparent antioxidant capacity of the extracts may be due to their better access to the DPPH sites which suggests that the extracts could contain compounds with small molecules rather than large molecules (Prior *et al.*, 2005). Antioxidant activity of oils or extracts has often been attributed to the presence of phenolic constituents especially spathulenol, carracrol and thymol (Aline, 2012) while Stephen *et al.* (2009) confirmed the presence of alkaloid, tannins, saponins, phytates and oxalates in *Khaya* species. Pooja *et al.* (2015) also opined that the presence of these phytochemicals in plant materials can act as natural antimicrobial or antioxidants. These results proved that extracts from *K. grandifoliola* plant parts may possess significant antioxidant properties and the medicinal benefits derived from their use may include prevention of oxidative damage and subsequent disease protection.

Table 2: DPPH free radical scavenging activity of *K. grandifoliola* extracts (LC_{50}).

Plant/ solvents	KG-A	KG-E	KG-W	Ascorbic acid
Leaf	$1.27±0.00^{b}$	$1.31±0.10^{b}$	$1.69±0.10^{b}$	$0.40±0.15^{a}$
Stem bark	$1.43±0.01^{c}$	$1.40±0.03^{c}$	$1.71±0.00^{c}$	$0.40±0.15^{a}$

Key: KG-E, *K. grandifoiliola* ethanol extract; KG-A, *K. grandifoiliola* acetone extract; KG-W, *K. grandifoiliola* water extract.

The antimicrobial activity of the extracts varied, it was shown to be dose dependent as greater inhibition of growth was observed as the concentrations of the extract increased (Bharati and Vidyasagar, 2012; Kashari *et al.*, 2013). The KG-ALE exhibited the highest activity, against *S. aureus* ATCC25923 followed by KG-ESE against *S. typhi* ATCC6539 while the least was observed in KG-W against *B. subtilis* ATCC6633. The plant extracts also showed significant antifungal activity for KG-ASE against *T. rubrum* and *A. flavus* and this was followed by KG-ESE against *T. rubrum.* The minimum inhibitory concentration (MIC) of the susceptible organisms ranged from 5 to 200 mg/mL of the efficacious extracts (Table 3).

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In all, KG-ALE and KG-ASE exhibited higher degree of antibacterial activity with MIC of 2.5 mg/mL against *S. aureus* and 5 mg/mL against *Streptococcus pneumoniae, S. typhi*, *E. coli* and *P. aeruginosa* respectively. KG-ESB exhibited MIC of 5 mg/mL against *B. subtilis*, *B. subtilis* ATCC6633, *S. aureus*, *S. aureus* ATCC25923, *S. pneumoniae*, *E. coli* and *E. coli* (ATCC25923) respectively. This may be due to the active substances in the extracts that were soluble in acetone and ethanol but not present in water extracts or were present in low concentrations.

Table 3: The MIC of *K. grandiofolia* extracts on the test microorganisms.

Test Organisms	KG-A		KG-E		KG-W	
	leaf	Bark	Leaf	Bark	leaf	Bark
B. subtilis	200	ND	200	5	10	15
B. subtilis (ATCC6633)	150	ND	150	5	10	15
S. aureus	2.5	2.5	7.5	5	25	15
S. aureus (ATCC25923)	2.5	2.5	7.5	5	25	15
S. pyogenes	100	ND	25	5	ND	ND
S. epidermidis	ND	ND	80	100	ND	ND
S. pneumoniae	5	ND	25	5	ND	10
E. coli	5	12.5	15	5	7.5	25
E. coli (ATCC25922)	5	12.5	15	5	7.5	25
E. faecalis	25	10	12.5	ND	15	200
K. pneumonia	ND	ND	ND	15	ND	ND
S. typhi	5	5	7.5	12.5	15	15
S. typhi (ATCC6539)	5	5	7.5	12.5	15	15
P. aeruginosa	15	5	5	15	25	7.5
A. flavus	ND	5	100	25	ND	10
C. albicans	ND	ND	100	ND	ND	5
C. albicans (10231)	ND	ND	80	ND	ND	5
C. glabrata	ND	ND	200	ND	ND	ND
C. neoformans	ND	ND	100	ND	ND	ND
T. rubrum	ND	7.5	7.5	5	ND	ND

Key: ND, Not detected

The values of MIC obtained from the KG-A and KG-E extracts of the plant materials were lower than those reported by Yusha'u (2011) and Essien *et al.* (2015) on similar medicinal plants. The effectiveness of *K. grandifoliola* against Gram-positive bacteria especially some antibiotics resistant strains of *Staphylococcus* corroborates the work of Stephen *et al.* (2009) who however, obtained a lower MIC of 0.4 mg/mL against *S. aureus*, *B. subtilis* and *K. pneumoniae.* Kubmarawa *et al.* (2008) had also reported the efficacy of *K. senegalensis* extracts on *S. aureus*, *S. pneumoniae*, *E. coli* and *P. aeruginosa.* The test extracts were more active against bacteria than fungi which is at variance with the works of Adekunle *et al.* (2003) who reported inhibition of *A. flavus*, *A. fumigatus*, *A. niger*, *Trichoderma viride* and *Trichophyton metagophytes.* Acetone and ethanol extracts were generally found to be more effective than water extract. Okigbo and Ogbonnaya (2006) posited that the differences observed in the plant extracts may be due to insolubility of active compounds in water as compared to acetone and ethanol. The presence of phytoconstituents like flavonoids, tannins, terpenoids, and phenols as reported by Ngoci *et al.* (2014) may have accounted for the antibacterial efficacy of the active plant extracts. This assertion is in agreement with the findings of Ishaku *et al.* (2012) and Geetha (2015) who confirmed the compounds as the most bioactive constituents in some members of the family Meliaceae. Abiodun *et al.* (2009) also asserted that the presence of α-pinene, β-pinene, limonene, citronellol, thugene and α-phellandrene in stem bark of *K. grandifoliola* may be responsible for the plant material antimicrobial activity. The results of the brine shrimp lethality bioassay are summarized in Table 4.

The plant extracts showed good brine shrimp larvicidal activity. Based on the results, the KG-E and KG-A were very toxic to the shrimps exposed for 24 h in dose dependent manner. Meyer *et al.* (1982) and Haq *et al.* (2012) posited that all LC_{50} values higher than 1000ppm are not significant while those within the range of 0-100 ppm are considered to be very toxic (Semuri *et al.*, 2012). The highest cytotoxic effect was exhibited by KG-ELE (0.67 ppm) followed by KG-ALE (8.07 ppm) and KG-ESE (0.91 ppm) while the KG-WLE and KG-WSE exhibited poor cytotoxic effect. Therefore, the plant materials may have some biological activity. The high potent activity of *K. grandifoliola* plant material extracts even at lower concentration suggests that these compounds could be toxic to tumor cells and may be developed further as anticancer drugs.

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Table 4: Percentage mortality of brine shrimps at different concentrations of *K. grandifoliola* extracts.

		KG-ALE			KG-ELE			KG-WLE		
Dosage (ppm)	Initial larvae	No. of survivors	No. of deaths	% mortality	No. of survivors	No. of deaths	% mortality	No. of survivors	No. of deaths	% mortality
1000	20	0	20.00 ±0.02^{b}	100	0	20.00 ±0.00^{b}	100	13	7.00 ±0.01^{a}	35
100	20	6	14.33 ±1.00^{b}	70	3	17.00 ±0.01^{b}	85	15	5.33 ±1.00^{a}	25
10	20	8	12.33 ±0.01^{b}	60	6	14.67 ±0.01^{b}	70	16	4.67 ±0.00^{a}	20
1	20	12	8.67 ±0.03^{b}	40	9	11.33 ±0.10^{c}	55	19	1.33 ±2.00^{a}	5
LC_{50}				8.07			0.67			1502
		KG-ASE			KG-ESE			KG-WSE		
1000	20	0	20.33 ±1.15^{b}	100	0	20.00 ±0.01^{b}	100	12	8.33 ±0.00^{a}	40
100	20	3	17.33 ±2.07^{b}	85	1	19.00 ±0.02^{b}	95	13	7.33 ±0.01^{a}	35
10	20	7	13.33 ±2.00^{b}	65	5	15.67 ±0.07^{b}	75	15	5.33 ±0.07^{a}	25
1	20	10	10.33 ±1.15^{b}	50	9	11.33 ±0.00^{b}	55	18	2.67 ±0.01^{a}	10
LC_{50}				1.00			0.91			1413

Values followed by different superscripts across each row are significantly different at $p \leq 0.05$.

CONCLUSION

The study revealed that *K. grandifoliola* plant materials may possess significant bioactive compounds which contributed to the pronounced antioxidant, antimicrobial and potent cytotoxic effect on brine shrimps. The KG-A and KG-E have great potentials as antimicrobial compounds especially in the treatment of infectious diseases caused by the susceptible organisms. Further work can be used as a lead to evaluate the active substances in the extracts for the development of drugs.

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