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Original Research Article

Comparative Evaluation of the Phytochemical Contents, Antioxidant and some Biological Activities of *Khaya grandifoliola* Methanol and Ethyl Acetate Stem Bark, Root and leaf Extracts

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ABSTRACT

Khaya grandifoliola extracts have been conventionally found to be effective in the treatment of wounds and ulcers. This study assessed the phytochemical content of *K. grandifoliola* methanol and ethyl acetate extracts and used *in vitro* bioassays for bioactivity testing to confirm the ethnomedicinal uses. Phytochemical content and bioactivity of *K. grandifoliola* ethyl acetate and methanol extracts; (stem bark, root, leaf) were assessed using spectrophotometric analysis with standard calibration curves for respective phytochemicals and *in vitro* bioactivity assays including MTT, free radical scavenging activity, lipopolysaccharide-activated RAW 264.7 macrophages assay for cytotoxicity, anti-inflammatory antioxidant, and antimicrobial activities. The polyphenolic content of methanol extracts (37.49±1.40 -53.57±1.50 mg GAEQ/100 mg) was the highest phytochemical content measured. The methanol root extract had the highest terpenoid concentration (35.78±2.14 mg LIN EQ/100 mg). Methanol stem bark and root extracts exhibited anti-inflammation activity at 200 µg/mL, potent antioxidant activity (IC_{50}; 38.68±5.09 µg/mL and 46.34±9.56µg/mL), and growth inhibition against *Staphylococcus aureus* (MIC; 1 and 2 mg/mg). Ethyl acetate root and leaf extracts inhibited the growth of *Streptococcus pyogenes* (MIC; 0.25 mg/mL). In conclusion, the high phenolic and terpenoid content of extracts which correlated positively with their biological activities could be the contributing factor to the biological effects such as wound healing of the extracts.

Keywords: *Khaya grandifoliola*, spectrophotometric analysis total phenolic content *Staphylococcus aureus, Streptococcus pyogenes*.

Introduction

The interactions of phytochemicals in medicinal plant extracts with the internal systems of living organisms contribute to the therapeutic value of medicinal plants. For instance, phenolic compounds have been found to interfere with quorum sensing, the regulatory mechanism of biofilm formation, and the differentiation of microorganisms resulting in the restriction of biofilm growth.[1, 2] The potency of plant extracts depends on the concentrations of phytochemicals which correlate positively with the potent biological activities of the extracts.[3]
Previous studies on the quantitative analysis of extracts have revealed that solvent extracts have high phenolic and flavonoid contents.

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For instance, Njayou *et al.*[4] estimated that the total phenolic content (TPC) of 100% methanol fraction of *K. grandifoliola* stem bark, assessed using the Folin Ciocalteu reagent and chlorogenic standards, was 32.72±0.60 CAE/g (chlorogenic acid equivalents per gram), whereas that of the methylene chloride/methanol (75/25; v/v) fraction was 38.40±0.03 CAE/g. Traore *et al.*[5] also assessed the TPC of the stem bark aqueous extract of *K. grandifoliola* with the same method but with Gallic acid standard calibration curve and the results revealed that the TPC of the extract was 186.75±12.76 mg GAE/mg.
Antimicrobial resistance of pathogens such as *S. aureus* causes a prolonged wound-healing process due to biofilm production. A traditional herbal practitioner (THP) of the Ho Municipal Hospital in the Volta region, Ghana, claimed that Tiger ointment® prepared using *K. grandifoliola* aqueous root extract is effective in the treatment of chronic wounds and ulcers.[6,7] Quartey *et al.*[6] reported that the ethanol stem bark extract of *K. grandifoliola* (KGE) promotes wound healing as all their test doses (KGE 15, 10, 5, and 1% and Drez® ointment, w/w) produced a significantly higher percentage of wound contractions than that of the untreated or negative control group.
Various ethnomedicinal uses of *K. grandifoliola* extracts exist, for example, the concoction of the stem bark is used for the treatment of convulsion, cough, stomach-ache, fever, threatened abortion, lumbago, and rheumatism traditionally. The ethnomedicinal uses of plant extracts have been confirmed through bioassay results obtained via various biological activity testing with both *in vivo* and *in vitro* experiments.[7-9]
This study was conducted to measure the phytochemical content of ethyl acetate and methanol extracts of *K. grandifoliola* plant parts and to relate the phytochemical content to the bioactivities against wound infections.

Materials and Methods

Solvents and reagents

Extraction was done with LiChrosolv® solvents (Merck, Germany) whilst standards and reagents, secured from Sigma Aldrich, were used for spectrophotometric analysis. L-Ascorbic acid, 1,1-Diphenyl-2-picrylhydrazy (Sigma, USA). PBS with and without Ca^{2+} and Mg^{2+} and trypsin-EDTA 3-(4,5-Dimethylthiazolyl-2-yl)-2,5-diphenyltetrazolium bromide and Melphalan (Tocris Bioscience South Africa). RAW 264.7 mouse macrophages and Vero African green monkey kidney cells, Cellonex (South Africa). Lipopolysaccharide, Griess reagent, and aminoguanidine, Sigma-Aldrich (St. Louise, MO, USA). Fluconazole, gentamicin sulfate, and vancomycin hydrochloride (Sigma, USA), Mueller-Hinton broth and Malt Extract broth and dimethyl Sulfoxide (Merck, USA), CellTiter-Blue® Reagent (Promega).

Plant material

The plant specimen of *Khaya grandifoliola C.DC*. was collected in Avatime Biakpa (Volta Region, Ghana) in December 2018. It was then identified, authenticated, and assigned a voucher Number, KNUST/HM/2020/L002, by Mr. Cliford Asare at the Herbarium Section, Department of Pharmacognosy, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Extraction and quantitative analysis

Shade-dried pulverized biomass of 15 g, 18 g, and 13 g of *K. grandifoliola* roots, stem bark, and leaves were sequentially macerated using 100% ethyl acetate and methanol with biomass to solvent ratio of 1:3 (w/v) for 48 hrs. as described by Traore *et al*.[10] with slight modification. Each extract was vacuum filtered using a Buchner funnel and concentrated to dryness using rotary evaporation (Buchi, Switzerland) at 50°C. The resulting extracts were collected, weighed, and stored at a temperature of 2°C for further analysis.

Quantitative analysis

Total alkaloid content was assessed using the bromocresol green (BCG) method with atropine standard as described by Shamsa *et al*.[11] with slight modification to suit the type of extracts used in this assay. Atropine was used as the reference standard to generate the calibration curve with a linear regression equation shown in Equation I.

$$y = 0.0551x + 0.0004;\ R^2 = 0.992 \qquad (I)$$

A stock solution of the standard and each extract (10 mg/1 mL) was prepared and 2 mL of each solution was transferred to a labelled separatory funnel. A phosphate buffer (5 mL) with a pH of 4.7 was added, followed by 5 mL of BCG. The mixture was then shaken well with 5 mL of chloroform twice, followed by vortexing resultantly, a yellow atropine-BCG complex in each separatory funnel. The yellow fraction was then collected and diluted with chloroform to 10mL volume. The absorbance of the yellow BCG- atropine complex in chloroform was measured at 470 nm against blank chloroform, using a UV-spectrophotometer. The total alkaloid content was estimated and expressed in milligram atropine equivalent per 100 mg of extract (mg ATPEQ/100 mg).

Total phenol content

The total phenolic content of each crude extract was assessed using the single electron transfer mechanism of the Folin-Ciocalteau reagent as described by Al-Ameri and Al-Shemari[12] with a few modifications. Gallic acid (Merck, US) was used as the reference standard to generate the calibration curve with a linear regression equation shown in Equation II.

$$y = 0.0214x + 0.0024;\ R^2 = 0.9832 \qquad (II)$$

An aliquot (1 mL) of Gallic acid solution (10 mg/1 mL distilled water) was added to 2.5 mL of Folin–Ciocalteu (10% v/v), 7.5 mL anhydrous sodium carbonate (7.5%) was then added resulting in a blue-coloured solution. The reaction mixture was incubated at 25°C for 40 mins and the absorbance read at a wavelength of 760 nm using a UV 2700 double beam spectrophotometer and recorded against reagent blank- methanol. The same procedure was repeated for each extract. The total phenolic content was estimated and expressed in milligrams of Gallic acid equivalent per 100 milligrams of extract (mgGAEQ/100 mg).

Total flavonoid content

The total flavonoid content of each extract was measured using the aluminium-chloride colorimetric assay as described by Agbo *et al*.[13] with slight modification.[13] Quercetin was used as the reference standard to generate the calibration curve with a linear regression equation shown in Equation III.

$$y = 0.0551x+0.0004;\ R^2=0.9928 \qquad (III)$$

An aliquot of standard solution (1 mL of 10 mg/mL standard solution) was pipetted into a 10 mL volumetric flask on 4 mL of distilled water followed by 0.3 mL of 1.45 $mol.dm^{-3}$ sodium nitrite. After 5 min 0.3 mL of 0.75 $mol.dm^{-3}$ aluminium chloride solution was added and at the 6th min, 2 mL of 1 $mol.dm^{-3}$ sodium hydroxide was added. The reaction mixture was further diluted to the 10 mL mark with distilled water. The absorbance was measured against the blank at 510 nm using a UV-visible spectrophotometer. The same procedure was repeated for each extract solution (1 mL of 10 mg/mL extract solution) The total flavonoid content was estimated and as expressed in milligram quercetin equivalent per 100 milligrams (mg GAEQ/100 mg).

Total terpenoids content

The total terpenoids content method was adopted from Ghora *et al.*[14] with minor modifications. The TTC of the extracts was estimated using a linalool calibration curve with a regression equation as shown in Equation IV.

$$y = 0.0204x+0.001;\ R^2 = 0.9731 \qquad (IV)$$

For each sample, a mass of 100 mg was dissolved in 10 mL of methanol (95% v/v). The mixture was shaken well and filtered. Half of the supernatant (5 mL) was mixed with 2 mL of chloroform and 3 mL of concentrated sulphuric acid. The mixture was allowed to cool in an ice bath for a maximum of 15 minutes. The mixture was then incubated at room temperature for 1.5-2 hrs. The supernatant was then decanted without disturbing the red–brown precipitate formed. A 1.5 mL of 95% (v/v) methanol was added to each precipitate collected and the mixture was shaken thoroughly until all the precipitation dissolved. The absorbance was measured against a blank at 538 nm using a UV-visible spectrophotometer. The total terpenoids content of each sample was estimated and expressed in milligram linalool equivalent per 100 milligrams of extract (mg LINEQ/100 mg).

Bioactivity testing

Antioxidant activity testing; DPPH radical scavenging assay

Antioxidant activity testing was assessed using single electron transfer mechanism DPPH as described by Njayou *et al*.[9] with slight modification. Two stock solutions, 0.1 x 10^{-3} $mol.dm^{-3}$ of DPPH, 1 mg/mL of ascorbic acid standard, and 1 mg/mL of each extract were prepared using 100% methanol. Aliquots of each extract solution (leaves, roots, and stem bark) and the ascorbic acid standard were prepared as serial concentrations (25-200 µg/mL) respectively, using methanol and absorbance was measured at a wavelength of 517 nm. A decrease in absorbance on the addition of test samples was used to calculate the antioxidant activity, expressed as the inhibition percentage (%) of DPPH using Equation IV below;

$$\%\ \text{Scavenged} = \left[\frac{ABSd - ABSa}{ABSd}\right] x100 \qquad (V)$$

Where ABS*d* is the absorbance of the negative control; ABS*a* is the absorbance of the standard ascorbic acid or extract. The IC_{50} value was computed by plotting the percentage of DPPH radical scavenging against the concentration of the sample showing the concentration of sample necessary to inhibit DPPH by 50%. All experiments were

carried out in triplicate. The radical scavenging activity of ascorbic acid served as a standard.

Cytotoxicity against Vero cells

Cytotoxicity was tested using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Vero African green monkey kidney cells (Cellonex, RSA) were cultured in DMEM low glucose cell culture medium supplemented with 10% FBS (complete medium). The cells were seeded at a density of 5000 cells/100 µL/well and incubated overnight at 37°C for attachment. Treatment for cytotoxicity assay was done in quadruplicate by adding an additional 100 µL of extract in a complete medium to achieve final concentrations of 50, 100, and 200 µg/mL. Cells were incubated at 37°C for 48 hrs. Melphalan at 25, 50, and 100 µM served as the positive control. After 48 hr treatment medium was removed. MTT was diluted in a complete medium to a final concentration of 0.5 mg/mL and added to wells using 100 µL aliquots. Cells were incubated at 37°C for 2 hrs. After incubation, the medium was aspirated, and the purple insoluble formazan product was dissolved by the addition of 100 µL DMSO. The absorbance was measured spectrophotometrically at 540 nm using a BioTek® PowerWave XS spectrophotometer.

Anti-inflammatory activity

The anti-inflammatory and viability assessment were conducted using the method described by Al-Sayed *et al.*[15] RAW 264.7 cells were seeded into 96-well plates at a density of 1 x 10^5 cells per well in low glucose DMEM:10% FBS and allowed to attach overnight. After removing the spent culture medium, the extracts (diluted in DMEM complete medium) were added to give final concentrations of 50, 100, and 200 µg/mL (50 µL per well at double the desired final concentration). To assess the anti-inflammatory activity, 50 µL of LPS-containing medium (final concentration of 500 ng/mL) was added to the corresponding wells. Aminoguanidine (AG) was used as the positive control for this experiment. Cells were incubated for a further 24 hr. To quantify NO production, 50 µL of the spent culture medium was transferred to a new 96-well plate and 50 µL Griess reagent was added. Absorbance was measured at 540 nm and the rest were expressed relative to the appropriate untreated control. A standard curve using sodium nitrite dissolved in a culture medium was used to determine the concentration of NO in each sample. To confirm the absence of toxicity as a contributory factor, cell viability was assessed using MTT. This was done by the removal of the remaining medium and treatments in each well and replacing it with medium containing 0.5 mg/mL MTT and further incubated for 30 minutes at 37°C. Thereafter, MTT was removed, and 100 µL DMSO was added to each well to solubilise the formazan crystals. Absorbance was read at 540 nm using a BioTek® PowerWave XS spectrophotometer (Winooski, VT, USA). MTT results were used to normalise nitrite production, eliminating false positive anti-inflammatory trends as a result of cytotoxicity.

Antimicrobial activity testing

The microorganisms used were clinical strains obtained from the NHLS in Port Elizabeth. *S. aureus*, *E. coli*, *P. aeruginosa* were grown in Mueller-Hinton (MH) broth (Merck, USA) and *C. albicans* was grown in Malt extract broth at 37°C. *S. pyogenes* was grown in Brain-Heart Infusion broth. One microorganism colony, from an overnight streak plate, was inoculated in broth (10 mL) and allowed to grow for 16 hrs (log growth phase) at 37°C. Gentamicin sulphate and vancomycin hydrochloride were used as positive controls against gram-negative and gram-positive bacteria, respectively. Fluconazole was used as positive control against *C. albicans*. Antibiotics were dissolved in dH_2O at stock concentrations of 2 mg/mL and filter sterilized (0.2 µM filter). Fluconazole was dissolved in DMSO at stock concentration of 5 mg/mL. Working concentrations were prepared in broth, depending on the antibiotics' MIC values. Antibiotic concentrations used based on bacteria/yeast were as follows: Bacteria: 0.064 – 0.005 mg/mL and *C. albicans*: 1– 0.0078 mg/mL (fluconazole). MH broth/Malt Extract/Brain-Heart infusion broth (50 µL) was added to all test wells (i.e., plant extracts and antibiotics), except for the highest plant extract and antibiotic concentration wells to which 100 µL of the working concentrations were added. Serial dilutions were prepared for the plant extracts (2 mg/mL - 125 µg/mL) and antibiotics (Vancomycin/gentamicin: from 64 to 0.25 µg/mL; fluconazole: 1 000–7.81 µg/mL). The cultures were assessed and adjusted to a 0.5 McFarland standard (absorbance at 600 nm = 0.08-0.1; equivalent to ~$1.5x10^8$ cells/mL) and 50 µL was added to each test well.

The following controls were prepared: Antibiotic/medium control (50 µL broth + 50 µL of highest antibiotic concentration); Extract colour control (50 µL broth + 50 µL of highest plant extract concentration); 4% DMSO control (50 µL broth + 50 µL 8% DMSO); Microorganism control (50 µL broth + 50 µL microorganism). Plates were sealed with microplate sealing tape and incubated at 37°C for 24 hrs. After treatment, 20 µL of CellTiter-Blue® Reagent was added to each well and incubated for 1 hr. Wells were observed for colour change, where a blue colour represented no growth (non-viable cells) and a pink colour represented growth (viable cells). MIC values were determined through visual inspection of the plate and reported as the lowest concentration where no colour change from blue to pink occurred.

Statistical analysis

Experiments were conducted in triplicates or quadruplicates for each assay. GraphPad Prism version 9 was used to compute the IC_{50} values. Statistical significance was concluded using the two-tailed student t-test.

Results and Discussions

The results of the maceration process of extraction gave out dark red course powder, reddish-brown coarse powder, and a dark green syrup as ethyl acetate root (EKR); stem bark (EKSB), and leaf (EKL) extracts. While the methanol extracts were thick sticky mahogany red (MKR); thick cherry red (MKSB); and green syrup (MKR). The percentage yield of 1.7%, 3.5%, and 4.2% of the roots, stem bark, and leaves (15 g, 18 g, and 13 g) was obtained using ethyl acetate whereas 12.5%, 21.1%, and 14.2% were recorded using methanol and the same mass of root, stem bark, and leaves respectively.

The spectrophotometric analysis of the extracts for the major phytochemicals was conducted based on Beer's law.[16] The results which confirmed the presence of alkaloids, phenolic compounds, flavonoids, and terpenoids in the extracts are summarized in Table 1.

Generally, the phytochemical content of methanol extracts is higher than that of ethyl acetate.

This could be attributed to the high content of polar compounds in the extracts.

Total alkaloid content

The total alkaloid content (TAC) of each extract was evaluated using the atropine (ATP) standard calibration curve (Figure 1) and Equation I, expressed in mg atropine equivalent per 100 mg of extract (mg ATPEQ/100 mg extracts).

The TAC ranged between 10.67±0.22 and 3.78±0.13 mg ATP equivalent/100 mg extract in MKSB and EKR respectively (Table 1).

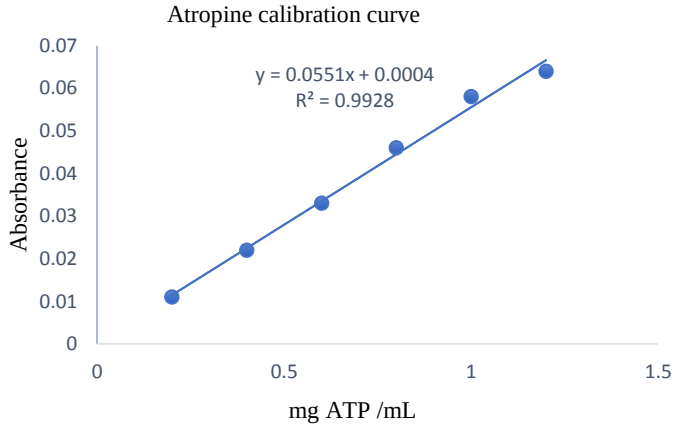

Figure 1: Atropine calibration curve used for total alkaloid content assessment

Total phenolic content

The analysis of the total phenolic content (TPC) of the extracts was based on the fact that in an alkaline medium, phenols reduce the mixture of phosphotungstic and phosphomolybdic acid present in the sample. Phenols reduce the Folin-Ciocalteu reagent to a blue coloured tungsten and molybdenum oxide chromophore which is proportional to the concentrations of phenolic compounds present in the extracts and evaluated using the gallic acid standard calibration curve (Figure 2) and Equation II.

The results were expressed in mg gallic acid equivalent per 100 mg of extract (mg GAEQ/100 mg).[17] All the plant parts retained high concentrations of phenolic compounds, with the highest accumulation recorded in the stem bark extract. This could be due to the presence of highly oxygenated compounds such as terpenoids especially limonoids in them.[17,18]

The TPC which was the highest phytochemical content recorded ranged between 53.57±1.5 and 8.38±1.08 mg GAEQ/100 mg extract in MKSB and EKR respectively. Elevated phenolic content in the extracts suggests that such extracts could minimize inflammation, provide complementary nutrition, and exert debriding effects, which occur during the proliferation phase of wound healing.[18,19] The phytochemical content of extracts has been found to correspond positively to their bioactivity according to the literature review. There is a positive correlation between the endpoint antioxidant activity and the total phenolic content of plant extracts.[19-21] The findings of this study have further emphasized this fact, thus the total phenolic content of methanol extracts correlates positively with antioxidant activity. The methanol stem bark which exhibited the highest total phenolic content of (53.57±1.50 mg GAEQ/100 mg extract) in the study, also showed the most potent antioxidant activity with the lowest DPPH IC_{50} (38.68±5.09 µg/mL). Extracts with high phenolic content (MKSB and MKR) were also found to be nontoxic, exhibiting moderate anti-inflammatory activity. These bioactivities of the extracts could contribute to wound healing confirming the ethnomedicinal uses of the *K. grandifoliola* extracts. These results are similar to the findings of Njayou *et al*.[9] which estimated the TPC of 100% methanol fraction of *K. grandifoliola* stem bark, using the Folin- Ciocalteu reagent method and chlorogenic standards to be 32.72±0.60 CAE/g (chlorogenic acid equivalents per gram), whereas that of the methylene chloride/methanol (75/25; v/v) fraction was found to be 38.40±0.03 CAE/g. Traore *et al*.[5] also assessed the TPC of the stem bark aqueous extracts of *K. grandifoliola* using the Folin-Ciocalteu reagent method and gallic acid standard calibration curve and the results revealed that the TPC of the extract was 186.75±12.76 mg GAE/mg.

Total flavonoid content

The total flavonoid content (TFC) was estimated based on the complexation of the vicinal hydroxyls of the flavonoid structures with $AlCl_3$. This reaction stimulates disruption of the hydroxyl groups based on the complexation of the vicinal hydroxyls of the flavonoid structures with $AlCl_3$, followed by spectrophotometric measurement due to the redshift displacement to form the flavonoid-Al^{3+} complex.[22,23] With the quercetin standard calibration curve (Figure 3) and Equation III, the TFC is estimated and expressed in mg quercetin equivalent per 100 mg of extract (mg QEQ/100 mg extracts).[16,23]

The TFC ranged between 10.26±0.92 and 4.22±0.91 mg QEQ/100 mg extract in MKSB and EKR respectively. The TFC in the methanol extracts was high with roots showing the highest flavonoid content while it was lowest in the leaf extract.

Total terpenoids content

The assay for approximating the total terpenoid content in crude extracts is based on a foundation of a chemical method in which linalool standard reagent reacts with concentrated sulphuric acid in a reaction that produces a brick red precipitate which is partially soluble in reaction mixture solution and chloroform but fully in methanol.[14] The absorbance of this brick red precipitate is therefore proportional to the concentration of the linalool. The total terpenoid content (TTC) of the extracts was estimated using a linalool calibration curve (Figure 4) with Equation (IV).

The results revealed that the highest TTC was that found in the methanol root extract which is 23.45±1.76 mg LIN EQ/100 mg, while the least was in the ethyl acetate leaf extract, 15.56±4.50 mg LIN EQ/100 mg. The high terpenoid content in the root extracts points to the confirmation of the presence of limonoids in the extracts.

Bioactivity testing

In vitro free radical scavenging activity

Antioxidants reduce oxidative stress which is one of the causes of diabetes and the major cause of diabetic foot ulcerations.[24,25] The results of the DPPH assay showed that the three plant parts of *K. grandifoliola* studied exhibited dose-dependent antioxidant potential. The dose-response data were used to obtain IC_{50} values (Table 2).

In vitro free radical scavenging activity

The endpoint antioxidant activity test was used to measure the antioxidant activity of medicinal plant extracts to assess the ability of the extracts to scavenge free radicals.[20,26] An antioxidant compound such as ascorbic acid donates an electron which is accepted by 1,1-diphenyl-2-picryhydrazine (DPPH) stable radical resulting in the reduction of the violet-colored DPPH solution to yellow-colored diphenylpicrylhydrazine radical.[19, 27] The results of the DPPH assay showed that the three plant parts of *K. grandifoliola* studied exhibited dose-dependent antioxidant potential. The dose-response data were used to obtain IC_{50} values (Table 2).

The mean IC_{50} for the ascorbic acid standard was found to be 48.06±1.52 µg/mL, while that of the methanol extracts were 38.68±5.09, 46.34±9.56 and 56.78±18.69 µg/mL for the stem bark, roots and leaves respectively, while the ethyl acetate extracts showed a lower measure of their effectiveness in scavenging free radicals with IC_{50} values of 67.90±3.03, 88.42±9.14 and 145.03±11.50 µg/mL in the same order of the plant parts as stated above.

Table 1: Total alkaloid, phenolic, flavonoid, and terpenoids contents of *Khaya grandifoliola* extracts

Extract	Total alkaloid content [mg ATPEQ/100 mg]	Total phenolic content [mg GAEQ/100 mg]	Total flavonoid content [mg QEQ /100 mg]	Total terpenoids content [mg LIN EQ/100 mg]
MKL	7.32 ± 0.14	37.49 ± 1.40	6.54 ± 0.55	10.16 ± 1.41
MKSB	10.67 ± 0.22	53.57 ± 1.50	10.26 ± 0.92	23.45 ± 1.76
MKR	8.49 ± 0.34	44.41 ± 0.69	9.58 ± 0.89	35.78 ± 2.14
EKL	4.22 ± 0.18	12.38 ± 1.04	5.16 ± 0.77	15.56 ± 4.50
EKSB	5.46 ± 0.14	24.34 ± 0.54	4.75 ± 0.48	16.75 ± 3.22
EKR	3.78 ± 0.13	8.38 ± 1.08	4.22 ± 0.91	17.82 ± 2.37

MKL: methanol *Khaya* leaves; MKSB: methanol *Khaya* stem bark; MKR: methanol *Khaya* roots; EKL: ethyl acetate *Khaya* leaves; EKSB: *Khaya* ethyl acetate stem bark; EKR: ethyl acetate *Khaya* roots; Values are expressed as mean ± SD of three experiments (n=3)

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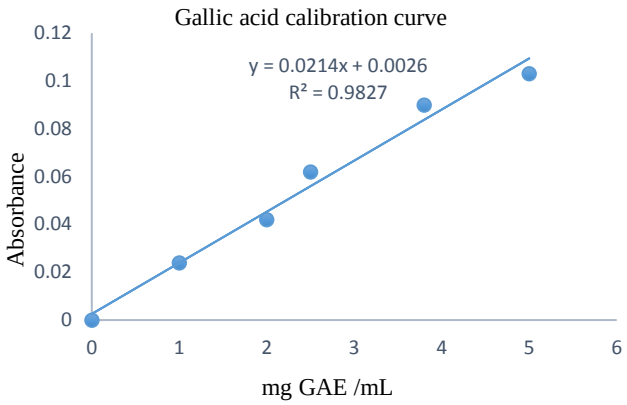

Figure 2: Gallic acid calibration curve used for total phenolic content assessment

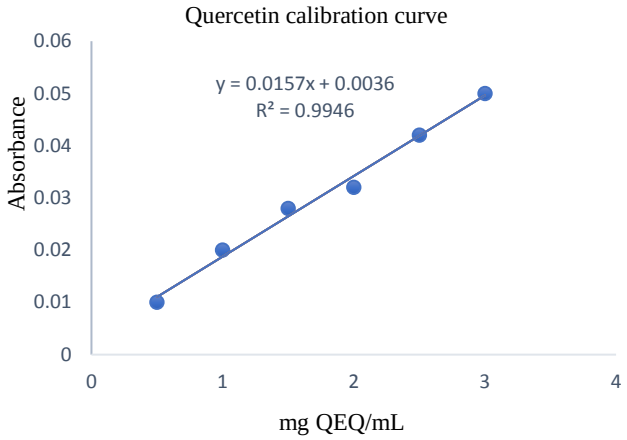

Figure 3: Quercetin calibration curve for total flavonoid content assessment

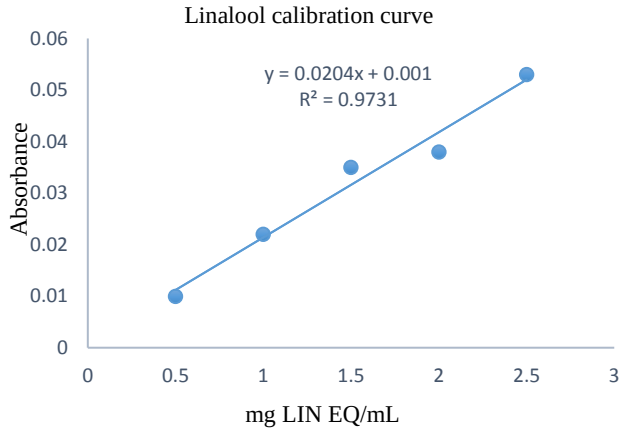

Figure 4: Linalool calibration curve for total terpenoids content assessment

MKSB and MKR exhibited potent antioxidant activity. Previous studies suggested that potent antioxidants are reactive oxygen species (ROS) scavengers that could improve wound contraction, increase blood vessel and fibroblast formation, and possibly terminate further tissue damage to improve normal wound healing.[28] These results were compared with the findings of Njayou *et al.*[9] which reported IC_{50} of methanol fraction of *K. grandifoliola* stem bark in the DPPH assay as 9.471±0.002 µg/mL.

Table 2: IC_{50} of methanol and ethyl acetate extracts of *K. grandifoliola*

Extract	IC_{50}	
	Methanol	**Ethyl acetate**
Stem bark	38.68 ± 5.09 µg/mL	88.42 ± 9.14 µg/mL
Leaves	56.78 ± 18.69 µg/mL	145.03 ± 11.50 µg/mL
Roots	46.34 ± 9.56 µg/mL	67.90 ± 3.03 µg/mL
Ascorbic acid	48.06 ± 1.52 µg/mL	48.06 ± 1.52 µg/mL

Cytotoxicity

The toxicity of ethyl acetate and methanol extracts was screened against Vero kidney cells using an MTT assay. The results are summarised in Figures 5A and B. The ethyl acetate leaf and root extracts were more toxic than the corresponding methanol extracts. Ethyl acetate stem bark and methanol stem, bark, and root extracts were non-toxic (Figures 5A and B).

Ethyl acetate leaf and root extracts showed a dose-dependent increase in the percentage of cell death by percentage; 90.91±1.86 and 54.58±10.73% respectively at a concentration of 200 µg/mL (Figure 5A).

The MTT assay revealed that the methanol stem bark and root extracts (MKSB and MKR) were non-toxic. The non-toxicity of extracts could imply the absence of toxicophore functional groups such as aromatic amines and carboxylic acids as well as various sites for hydroxylation in the compound.[29] Non-toxic MKSB and MKR could be useful in the treatment of skin infections and stimulate cell proliferation which helps to protect and maintain skin elasticity. The toxic extracts could not support skin cell proliferation.[30] Previous findings revealed that stem bark and leaf extracts were toxic. For instance, Oyewale and his coworkers[31] found out that *K. grandifoliola* leaf and stem bark extracts exhibited lethality against brine shrimp larvae with LC_{50} values ranging from leaf extracts (0.67 to 1502 µg/mL) and stem bark extracts (0.91 to 1431 µg/mL).

Anti-inflammatory activity

The results revealed that the methanol root extract (MKR) decreased lipopolysaccharide-induced nitric oxide (NO) production in RAW 264.7 macrophages at 200 µg/mL while the ethyl acetate stem bark extract (EKSB) moderately reduced NO production at all tested concentrations. MKSB and MKL had no significant effect on NO levels. The remaining extracts, EKR and EKL, were toxic to the macrophages (Figure 6).

The results revealed that MKR decreased lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW 264.7 macrophages at 200 µg/mL while EKSB moderately reduced NO production at all tested concentrations. Control in the production of inflammatory mediators such as ROS, NO, and pro-inflammatory cytokines may speed up the later stages of the wound healing process. A study on carrageenan and serotonin–induced paw edema and xylene-induced ear edema using RAW 264.7 macrophages, revealed that the methanol stem bark extract of *K. grandifoliola* significantly inhibited the carrageenan-induced paw edema from the first hr to the fifth hr at a dose of 200 mg/kg while a dose of 500 mg/kg significantly inhibited the carrageenan-induced paw edema after 3 hrs of carrageenan challenge.[32] Also, an *in vitro* anti-inflammatory effect of crude extracts of glycosides and aglycones obtained from drugs of *K. grandifoliola* using membrane stabilization and proteins protection tests revealed that the stem bark extracts possess anti-inflammatory activity.[33] However, the anti-inflammatory activity of the root extracts is first reported in this paper.

MIC of extracts and isolated compound screened against tested organisms

The minimum inhibitory concentration (MIC) is a qualitative assessment estimated by a colour change from blue to pink/purple due to the reduction of the CellTiter-Blue metabolic dye by viable bacteria. Both methanol and ethyl acetate extracts of the stem bark and root have been shown to possess antibacterial activity against gram-positive *S.*

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aureus and *S. pyogenes*. The growth of *S. aureus* is the most prevalent pathogen implicated with skin and wound infections worldwide due to its biofilm formation leading to antimicrobial resistance.[34] The results generated from the analysis of the crude extracts are summarized in Table 3.

The growth inhibition of *S. aureus* by MKSB and MKR at MIC of 1 mg/mL is quite promising as these extracts could promote wound healing. MKL, EKL, and EKR also exhibited very promising growth inhibition against *S. pyogenes* at an MIC of 0.25 mg/mL. The susceptibility of *S. pyogenes* to these extracts is a strong indication that the extracts could completely inhibit the growth of the bacteria, allowing the wound-healing process to proceed. These results were compared to the findings of Stephen *et al.*[35] who reported that the ethanol stem bark extract of *K. grandifoliola* inhibited *S. aureus* growth at an MIC = 0.4 mg/mL. The difference in the MIC value may be due to the bioassay method employed (agar dilution vs broth dilution methods).

MKSB, MKR, and EKSB exhibited growth inhibition against *S. aureus* at an MIC of 1 mg/mL. The growth of *S. pyogenes* was suppressed by both ethyl acetate and methanol extracts with MIC values ranging from 0.25–2 mg/mL. MICs of the gram-negative bacteria (*E. coli* and *P. aeruginosa*) and *C. albicans* were greater than the highest tested concentration of 2 mg/mL.

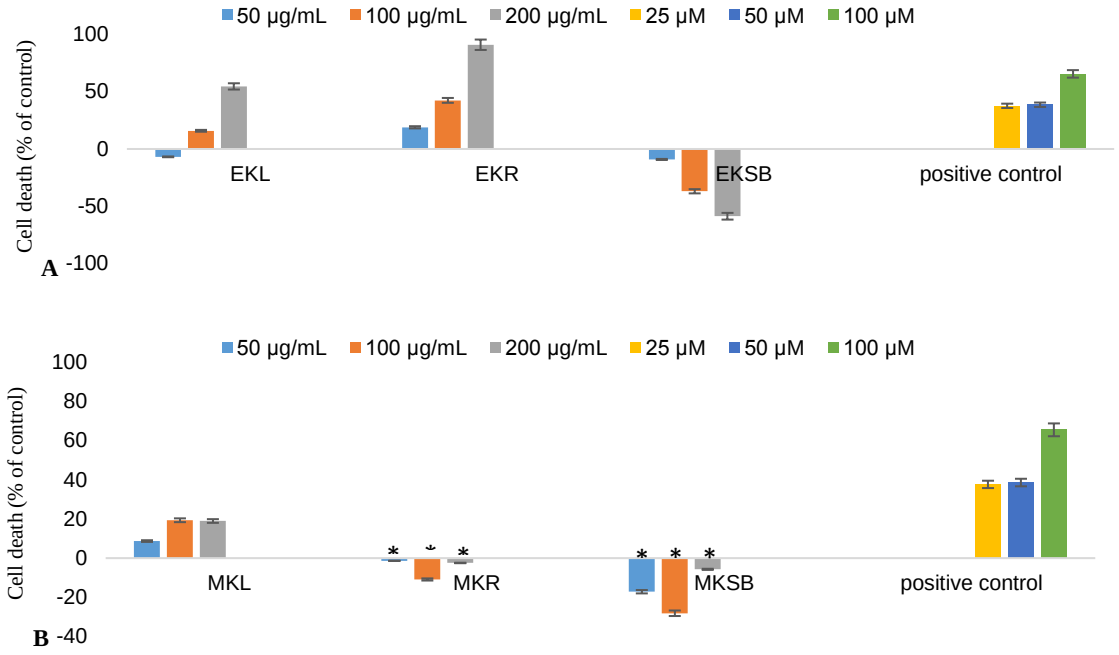

Figure 5: Cytotoxicity assessment of extracts and positive control melphalan against Vero cells (A) EtOAc extracts [EKL, EKR, EKSB], (B): MeOH extracts [MKL, MKR, MKSB]. Data points represent the mean ± SD of quadruplicate determinations. *Indicates statistical significance $p < 0.05$ using two-tailed Student's *t*-test compared to control cells.

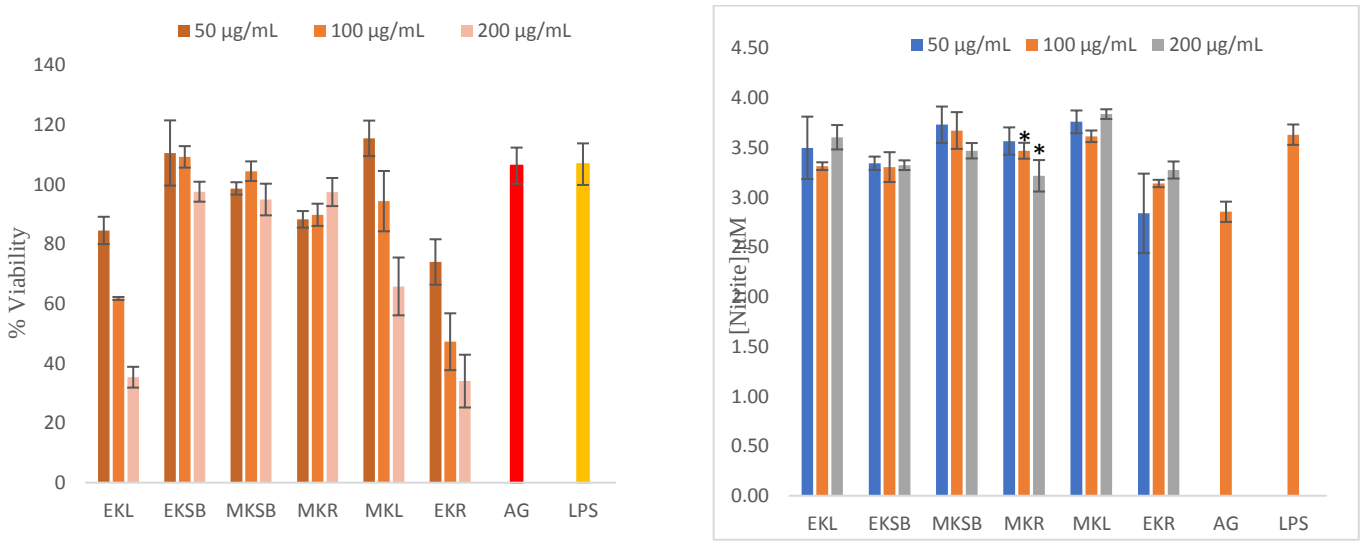

Figure 6: Cytotoxicity as reflected by the MTT assay (left) and anti-inflammatory activity normalised using MTT results (right) of *Lantana camara* extracts on RAW 264.7 macrophages

MKL: methanol *Khaya* leaf extract; MKR: Methanol *Khaya* root extract; MKSB: methanol *Khaya* stem bark extract; EKL: ethyl acetate *Khaya* leaf extract; EKR: ethyl acetate *Khaya* root extract; EKSB: ethyl acetate *Khaya* stem bark extract. Data points represent the mean ± SD of quadruplicate

determinations. Statistical significance compared to LPS- activated macrophages (*p < 0.05, and **p< 0.01) respectively, (n = 4) using two-tailed Student's *t*-test

Table 3: MIC of extracts and isolated compound screened against *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans*

Sample ID	MIC [mg/mL]				
	S. aureus	***S. pyogenes***	***E. coli***	***P. aeruginosa***	***C. albicans***
EKL	> 2	0.25	> 2	> 2	> 2
EKR	> 2	0.25	> 2	> 2	> 2
EKSB	1	2	> 2	> 2	> 2
MKL	> 2	0.25	> 2	> 2	> 2
MKR	1	2	> 2	> 2	> 2
MKSB	1	2	> 2	> 2	> 2

EKL: ethyl acetate *Khaya* leaf extract; EKR: ethyl acetate *Khaya* root extract; EKSB: ethyl acetate *Khaya* stem bark extract; MKL: Methanol *Khaya* leaf extract; MKR: methanol *Khaya* root extract; MKSB: Methanol *Khaya* stem bark extract

Conclusion

The outcome of the study indicates that the *K. grandifoliola* methanol stem bark and root extracts had the highest total phenolic content. The cytotoxicity results revealed that the extracts were non-toxic to human cells and had potent radical scavenging activity. Promising growth inhibition against *S. aureus* at a low concentration was also observed for these extracts. The methanol root extract exhibited moderate anti-inflammatory activity and growth inhibition *S. pyogenes* at a low MIC confirming. These properties could all contribute to the wound-healing activity of these extracts, confirming their ethnomedicinal uses. Although ethyl acetate leaf and root extracts were toxic, their MICs against *S. pyogenes* were potent and could be explored for antimicrobial agents. Ultimately, methanol root and stem bark extracts exhibit antimicrobial activities which contribute to their abundant biological activities including a wound healing potential.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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