Full Length Research Paper

Evaluating the antibacterial, antioxidant and wound healing properties of the stem bark extract of Khaya grandifoliola (Welw) CDC (Meliaceae) (African mahogany)

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The study was conducted to evaluate the antibacterial, antioxidant, and wound healing properties of the stem bark of Khaya grandifoliola (Welw) CDC (Meliaceae). A preliminary phytochemical screening conducted showed the presence of tannins, alkaloids, saponins, reducing sugars, flavonoids, terpenoids, and phenols in the stem bark (KG) as well as its ethanol extract (KGE). The antibacterial activity of KGE was evaluated using agar well diffusion method against Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, and Klebsiella pneumoniae. In-vitro antioxidant activity of KGE was also evaluated using the total phenolic content, DPPH radical scavenging activity, and total antioxidant capacity assays. In the wound healing activity test, topical formulations of varying concentrations of KGE (1-15% w/w) with Emulsifying Ointment BP were used in an excision wound model involving Wistar albino rats. KGE demonstrated in-vitro antibacterial activity against all test microorganisms in a dose-dependent manner. It also showed good antioxidant effects, with a strong correlation between the antioxidant capacity and phenol content (r = 0.9898); indicating that the observed effects may be due to the phenolic compounds initially detected. KGE showed significant wound healing effects as compared to the untreated group (p < 0.05). Additionally, the observed wound healing effects from the adopted doses were showed to be comparable (p > 0.05). In effect, the smallest dose was as effective as the highest dose. These outcomes showed that KG was effective as an antibacterial and antioxidant agent, and a wound healing promoter, justifying its reported traditional uses for infections and wound management.

Key words: Wound healing activity, antibacterial agent, antioxidant effects, tannic acid, medicinal plant, African mahogany.

INTRODUCTION

A wound is defined as the loss or breaking of cellular and anatomical or functional continuity of the skin, mucous membrane or tissue surface (Agyare et al., 2013; Kokane et al., 2009) which can be caused by physical, chemical and microbiological sources, and/or immunological mechanisms (Raina et al., 2008). There are several types and severities of wounds (Builders and Builders, 2016). Those that heal slowly tend to be infected if not taken...
Khaya grandifoliola (Welw.) CDC of the family Meliaceae, also known as African mahogany (Ojokuku et al., 2010) is a tree found in most parts of Africa, including Benin, the Democratic Republic of Congo, Ivory Coast, Ghana, Guinea, Nigeria, Sudan, Togo, and Uganda (Ojokuku et al., 2010). The tree has valuable use in the timber industry. It is usually grown in plantation within its natural habitat. In Benin, Nigeria, Congo, and Senegal, it has also been used for a wide range of medicinal purposes, including the treatment of convulsion, cough, stomach ache, fever, threatened abortion, rheumatism, dermatomycosis, malaria fever (Ojokuku et al., 2010), lumbago, gastric pains, worm infestation (Stephen et al., 2009) among others. Extracts of the bark have shown antiplasmodial activity in mice infected with Plasmodium berghei (Agbedahunsi et al., 1998), hypoglycemic, hypoproteinemic and hypocholesterolemic effects (Bumah et al., 2005), as well as cytoprotective effects against gastric ulcerations (Sandrine et al., 2016). Although some studies have demonstrated the antioxidant (Njayou et al., 2013; Sandrine et al., 2016; Yunga et al., 2018) and antimicrobial effects (Stephen et al., 2009) of the plant, very limited evidence exists on its wound-healing effects. In Ghana, the people of Bimbilla in the Northern region use the plant for treating wounds. The same has been reported in the Oyo State of Nigeria (Rafiu and Sonibare, 2017). The current study was thus premised on the afore-mentioned medicinal benefits of the plant, and it was proposed that K. grandifoliola (KG) may possess wound healing effects in addition to antioxidant and antibacterial effects.

MATERIALS AND METHODS

Chemicals, reagents, reference compounds, and equipment

All the chemicals and reagents used were of analytical grade. Chemicals used included ethanol (98% v/v, GPR, BDH, Poole, UK), methanol (≥ 99.9%, VWR Chemicals, UK), chloroform (99.9% v/v, AR, Marek, UK), normal saline (N/S) infusion (0.9% w/v, Sanbao (GH) Pharmaceuticals Ltd, Ghana), Fehling’s solution A and B (GPR, BDH, Poole, UK), sulphuric acid (98.5% v/v, GPR, BDH, Poole, UK), hydrochloric acid (36% v/v, GPR, BDH, Poole, UK), Sodium hydroxide 96% v/v (GPR, BDH, Poole, UK), Dragendorff’s reagent (50% v/v, AR, Marek, UK), ammonia solution (30% v/v, AR, Marek, UK) and iron (II) chloride (97.9% v/v, GPR, BDH, Poole, UK). The reference compounds used included Azithromycin (Ernest Chemist Limited, Ghana), 2, 2-Diphenyl-1-picyrylhydrazyl (90% w/w, Sigma Aldrich, Darmstadt, Germany), Ascorbic acid (Ernest Chemist Limited, Ghana) and Tannic acid (Sigma Aldrich, Darmstadt, Germany). Direz® ointment (Indus Life Sciences Pvt. Ltd, India) was used as a positive control drug. Equipment used included Rotary evaporator-R 10 (Buchi, Germany) and UV-spectrophotometer (Cecil 2000 series, Basildon Ltd., UK).

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Plant material collection and processing

The stem bark of KG was collected in Bimbilla in the Northern Region of Ghana (8°51’33.444”N 0°3’29.772”E) by a local herbalist in January 2019. The plant material was then authenticated at the Department of Medicinal Chemistry and Pharmacognosy in the School of Pharmacy at Central University, Ghana. A voucher specimen (CU/PD/2019/SB003) was deposited in the school’s herbarium. The stem bark was cleaned of foreign matter, chopped into smaller pieces, and sun-dried for a week. The dried material was then coarsely powdered using a mechanical grinder.

Extract preparation

The extract was prepared following described methods (Singh, 2008). 500 g of pulverized KG was cold macerated with 1000 mL of ethanol with regular shaking for 72 h. The mixture was decanted, pre-filtered with cotton wools, and finally with Whatman No. 1 filter paper. The filtrate was concentrated in-vacuo using Rotary evaporator-R 10 and then air-dried to obtain the final extract (KGE; %yield = 6.16% w/w). KGE was then stored in a desiccator for future use.

Phytochemical screening

KG and KGE were subjected to preliminary phytochemical screening (Orman et al., 2015; Mireku-Gyimah et al., 2018). Phytochemicals tested included tannins, saponins, reducing sugars, anthracone glycosides, cardiac glycosides, cyanogenetic glycosides, steroids, phenols, terpenoids, alkaloids and flavonoids.

In-vitro antibacterial activity

Microorganisms

Reference strains of the microorganisms, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 8032 were employed for the studies. These organisms were obtained from the Department of Microbiology, Korle-Bu Teaching Hospital, Accra, Ghana. The isolates were identified by biochemical tests (Kar., 2008). Stock cultures of the organisms were maintained in nutrient agar plates at 4°C and sub-cultured in nutrient broth at 37°C for 24 h before the experiment.

Antibacterial activity test

Agar well diffusion method as described by Srinivasan et al. (2001) was used to evaluate the antibacterial activity of KGE (at concentrations, 20, 10, 5, and 2.5 mg/ml) against the test organisms. Azithromycin (AZT, at 1 mg/mL) was used as a positive control. Plates of nutrient agar were seeded with cultures and wells were cut with a sterile cork borer (size = 6 mm). 0.5 ml each of KGE solutions (dissolved in distilled water) were pipetted into the wells, allowed to diffuse at room temperature for 1 h, and incubated at 37°C for 24 h. The activity of KGE was evaluated by measuring the zones of inhibition from each well. The experiment was carried out in duplicate and the mean diameter of the zones of inhibition was calculated.

In-vitro antioxidant assays

The antioxidant activity of KGE was assessed using the DPPH free radical scavenging, total antioxidant capacity, and total phenol content *in-vitro* models.

DPPH radical scavenging assay

The procedure adopted involved the use of the stable radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ascorbic acid as the standard (Amponsah et al., 2016; Ayensu and Quartey, 2015). A solution of DPPH (20 mg/L), as well as serial concentrations of KGE (0.0610 - 1000 µg/ml) and ascorbic acid (0.0610 - 500 µg/ml), were prepared in methanol. 1 ml each of KGE solutions was added to 3 ml of the DPPH solution and incubated for 30 min at 37°C in the dark. Similarly, 1 mL of ascorbic acid solutions was also added to 3 mL of the DPPH solution and incubated for 30 min at 37°C in the dark. The absorbances for resultant mixtures were taken at 517 nm with a UV-spectrophotometer using methanol as blank. Triplicate determinations were carried out. Radical scavenging activity for each test solution was determined using the formula:

\[
\%\text{Scavenging} = \frac{\text{Absorbance of blank} - \text{Absorbance of test solution}}{\text{Absorbance of blank}} \times 100\%
\]

A non-linear regression fit of the scavenging activities of KGE and ascorbic acid was adopted to estimate the EC50 of tested agents.

Total phenol content

The method adopted was as described in the literature (de Oliveira et al., 2012). The procedure involved the use of 0.5 N Folin-Ciocalteu reagent and tannic acid as a reference standard. Serial concentrations of tannic acid (7.8125 - 250 µg/ml) were prepared and 0.5 ml of each was added to 0.1 mL of Folin-Ciocalteu reagent and incubated at room temperature for 15 min. 2.5 ml of saturated sodium carbonate was then added and re-incubated for a further 30 min at room temperature. The absorbances for the resultant solutions were taken at 760 nm using a UV-spectrophotometer. Triplicate determinations were carried out. Selected concentrations of KGE (32.25, 62.50, and 250 µg/ml) were prepared and taken through the same procedure, to also record triplicate absorbances. A linear regression model (Figure 2b) was developed from the outcomes of the reference standard and this was used to estimate the total phenol content of KGE as expressed in terms of tannic acid equivalent (mg/g TA) (Table 2). All tests were done in triplicates.

Total antioxidant capacity

The procedure adopted was as described in the literature (Prieto et al., 1999) and involved the use of ascorbic acid as a reference standard and a standard reagent mixture (SRM) prepared from 0.6 M sulphuric acid, 28 mM disodium phosphate and 4mM ammonium molybdate. Serial concentrations of ascorbic acid (1.953 – 125 µg/ml) were prepared and 1 ml of each was added to 3 ml of SRM, incubated at 95°C for 24 h, cooled at room temperature and centrifuged for 10 min. The absorbance of the supernatant liquid of the various concentrations was measured at 695 nm with a UV-spectrophotometer. Triplicate determinations were carried out. Similarly, selected concentrations of KGE (31.25, 62.50, and 125 µg/ml) were prepared and taken through the same procedure to also record triplicate absorbances. A linear regression model (Figure 2c) was developed from the outcomes of the reference standard and this was used to estimate the total antioxidant capacity of KGE as expressed in terms of ascorbic acid equivalent (mg/g Vit C) (Table 2).
Wound healing activity

Animals

The wound healing studies were performed according to internationally accepted principles for laboratory animal use and care (NIH, 2011) and with due permission from Animal Ethics Committee (FPBS-AEC/CA02/19) in the Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. Healthy Wistar albino rats of both sexes, weighing 80-150 g, were procured from the Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana and were individually housed and maintained under standard environmental conditions of temperature (24.5 ± 0.50°C) and humidity (50 ± 5%) and 12 h light/dark cycle. They were fed with commercial pellet diet (GAFCO, Ghana) and clean autoclaved water ad libitum. Animals were allowed to acclimatize for a week before the test was initiated. They were then randomized into 6 groups of fives with labels, A, B, C, D, E, and F, and weighed. Groups A, B, C, and D were dedicated to four concentrations of KGE (that is, 15, 10, 5, and 1% respectively). Groups E and F were designated for negative and positive control groups, with the negative control group being untreated whiles the positive control group received treatment with Drez® ointment.

Sample preparation

Topical aqueous creams (200 g each) of varying concentrations of KGE (that is, 1, 5, 10, and 15% w/w) were prepared using Emulsifying Ointment BP (BPC, 2012) as a base.

Excision wound model

The excision wound model as described by Boakye et al. (2018) was adopted. The dorsal fur of the animals was gently shaved to expose the skin and cleaned with 70% v/v ethanol. The animals were anesthetized with chloroform and excision wounds of about 20 mm in diameter carefully created under aseptic conditions. The wounds were cleaned with a wad of cotton and N/S. The animals were closely monitored for any infection and those showing signs of infection were excluded from the study and replaced accordingly. The wound diameters were recorded after 24 h and wounds were subsequently treated topically, with daily applications of KG, (15, 10, 5 and 1%) and Drez® ointment after cleaning with N/S, in the test groups as earlier described. Wound contractions were calculated from the wound diameters recorded every 72 h for the next 15 days.

\[
\text{\% wound contraction} = \frac{\text{wound area on day } 1 \text{ - wound area on day } n}{\text{wound area on day } 1} \times 100\% \tag{2}
\]

where n = number of days 3rd, 5th, 7th, 9th, 11th, 13th, and 15th day.

Data analysis

Data analysis was performed using GraphPad Prism for Windows (version 8.0.2, GraphPad Software Inc, San Diego, USA, 2019). Data were described using the descriptive parameters, means, standard deviations, and standard error of means. Analysis of variance (ANOVA) followed by Tukey’s and Dunnett’s post-hoc tests was carried out to compare the means of outcomes between and within test groups at a 95% confidence level. Data from reference compounds in the total antioxidant capacity and phenol content tests were analyzed using linear regression by the least-squares method to develop linear regression models used to estimate the antioxidant capacity and phenol content of KGE. Data from the radical scavenging test were fitted to a non-linear regression model (Equation 3), to determine concentrations responsible for 50% of the maximum antioxidant activity (EC50) for both KGE and ascorbic acid. The correlation between total antioxidant capacity and phenol content was evaluated by determining the Pearson’s correlation coefficient for the mean absorbances obtained from the optimized concentration ranges for the above-mentioned tests.

\[
y = \frac{bottom + (top - bottom)}{1 + 10^{(x - \log EC_{50})}} \tag{3}
\]

Where ‘y’ is the response and ‘x’ is the logarithm of concentration of the KGE.

RESULTS AND DISCUSSION

The phytochemical screening showed the presence of tannins, alkaloids, saponins, reducing sugars, flavonoids, terpenoids, and phenols in both KG and KGE (Table 1). Anthracene and cyanogenetic glycosides were however absent in both. The presence of tannins, saponins, reducing sugars, alkaloids confirmed the outcomes from previous phytochemical studies on KG (Falodun et al., 2009; Ibrahim et al., 2006; Ojokuku et al., 2010; Stephen et al., 2009). On the contrary, whereas anthracene and cardiac glycosides were not detected in the current study, they were shown to be present in previous studies (Ibrahim et al., 2006; Ojokuku et al., 2010; Stephen et al., 2009). Also, while flavonoids were present in the current study, Ojokuku et al. (2010) and Ibrahim et al. (2006) reported their absence. These differences have been argued to be as a result of differences in geographical locations of the plants (Liu et al., 2016) as well as effects from the different modes of handling and preparing the plant samples (Tiwari and Cummins, 2013). Comparatively, KG, and KGE contained similar phytoconstituents except for cardiac glycosides and steroids, which were detected in KG but not in KGE. This obviously could be attributed to the ethanol solvent used; which has a high affinity for polar compounds than non-polar compounds (Dhawan and Gupta, 2016; Truong et al., 2019).

Most of these phytochemicals have been shown to possess antibacterial activity. For example, in a review conducted by Barbieri et al. (2017), the authors enumerated several phytochemicals classified under alkaloids, sulfur-containing phytocompounds, terpenoids, carotenoids, and polyphenolic compounds, possessing antibacterial effects; with some exerting effects against resistant and multi-resistant microorganisms (Barbieri et al., 2017). It could thus be inferred that the presence of these compounds confers antimicrobial properties on KGE, as documented in the literature (Galani et al., 2016; Okpe et al., 2019; Stephen et al., 2009). In the current study, KGE demonstrated dose-dependent antibacterial activities against the test organisms, S. aureus, E. coli,
Table 1. Results of the phytochemical screening of both the powdered plant sample and extract.

| Test          | Powdered plant sample (KG) | Plant extract (KGE) |
|---------------|-----------------------------|---------------------|
| Tannins       | +                           | +                   |
| Reducing sugars| +                           | +                   |
| Saponins      | +                           | +                   |
| Anthracene glycosides | -               | -                   |
| Cyanogenic glycosides | -        | -                   |
| Cardiac glycosides  | +                   | -                   |
| Alkaloids     | +                           | +                   |
| Flavonoids    | +                           | +                   |
| Steroids      | +                           | -                   |
| Terpenoids    | +                           | +                   |
| Phenols       | +                           | +                   |

[ + ] detected; [ - ] not detected.

Figure 1. Results from the antibacterial activity of KGE. [A] - Dose-dependent zones of inhibition from KGE on different microorganisms. Data expressed as Mean ± SD (N = 2). Two-way ANOVA analysis followed by Tukey's and Dunnett’s multi-comparison tests was carried out to determine the significance of differences for the different concentrations of KGE against the test microorganisms. [B] - Total Area under curve calculations of the zones of inhibition for KGE against test microorganisms. Data expressed as Mean ± SD (N = 2). One-Way ANOVA analysis followed by Tukey’s post-hoc test was used to compare the antibacterial activities against test organisms. Levels of significance were shown as *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001.

and K. pneumoniae. In other words, as the concentration of KGE increased, the zones of inhibition became larger (Figure 1a). The inhibitions produced from similar test concentrations of KGE against the test organisms were shown to be comparable ($F_{(12,20)} = 0.4254; p = 0.9345$, Figure 1). This was further confirmed from their Area under Curve calculations (AUCs), which were comparable for all the test organisms ($F_{(3,4)} = 0.1858; p = 0.9009$; Figure 1b). However, KGE’s activities against the test organisms were lower than that of the standard drug AZT (1 mg/ml) ($p < 0.0001$; Figure 1a).

KGE also demonstrated good antioxidant effects (Figure 2 and Table 2). The DPPH scavenging test assessed the ability of KGE to terminate free radical initiated reactions, which occur during oxidative stress conditions. The total antioxidant capacity measured the phytochemical constituents with antioxidant properties while the total phenolic content measured all phenolic and polyphenolic phytochemicals in the plant extract. The higher the values, the higher the antioxidant effect.
Figure 2. Results from Antioxidant activity tests. (A) Non-linear regression fit from DPPH Radical scavenging activity of KGE as compared to the standard drug, Ascorbic acid. Data were expressed as Mean ± SD (N = 3). (B) Linear calibration model developed from Tannic acid used to estimate the Total Phenol Content of KGE. (C) Linear calibration model developed from Ascorbic acid used to estimate the Total Antioxidant Capacity of KGE.

| Antioxidant test | Results |
|------------------|---------|
| DPPH Assay (IC₅₀) (N = 3) | KGE = 5.481 µg/ml |
| TAC (N = 9) | 1028 ± 68.02 mg/g Vit C |
| TPC (N = 9) | 999.7 ± 5.901 mg/g TA |
| Ascorbic acid = 0.7369 µg/ml |

(Barku et al., 2013). Thus, KGE was shown to have a relatively higher antioxidant capacity and phenol content when compared to other types of extracts from the same plant reported elsewhere (Njayou et al., 2013). The high correlation between the total phenolic content and the antioxidant capacity (Table 3) could mean that the antioxidant activity of KGE was mainly due to the phenolic compounds like flavonoids, terpenoids and tannins present (Güçin et al., 2010; Liu et al., 2009; Pietta, 2000; Villaño et al., 2007). Most of these phytochemicals possess aromatic rings in their structures and are thought to react with free radicals, resulting in the delocalization of the gained electron over the phenolic structure, leading to stabilization by resonance effect. This phenomenon disrupts the free radical reaction chain. Thus, a high amount of these phytochemicals in a plant shows a high antioxidant activity (Lee et al., 2017).

KGE was established to possess wound healing effects, as most of the test doses of KGE produced % contractions significantly higher than that of the untreated or negative control group.
Table 3. Pearson’s Correlation coefficients (r) between test antioxidant parameters.

|               | DPPH assay | TAC  | TPC  |
|---------------|------------|------|------|
| DPPH assay    | -          | -    | -    |
| TAC           | -0.7177    | -    | -    |
| TPC           | -0.4363    | 0.9898* | -    |

*Strong correlation observed. p-value = 0.001.

Figure 3. Results from in-vitro wound healing activity of KGE. (A) Line plot of wound contractions recorded from the test samples over 15 days of treatment as expressed as a percentage. Data expressed as Mean ± SD (N = 5). (B) Wound contraction recorded from the test samples as expressed as Total Area under Curve for treated animals in each test group. Data expressed as Mean ± SD (N = 5). One-Way ANOVA analysis followed by Tukey’s posthoc test was carried out to determine the significance of the difference in the AUC recorded for the test groups. AUCs of activities from KGE (15, 10, 5 and 1%) were compared among themselves, and with negative and positive controls. Level of significance was shown as *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001.

(Figure 3a and 3b). It is thought that the observed antioxidant and antibacterial activities as earlier discussed could contribute to and/or promote KGE’s wound-healing effects. The phytochemicals present in the plant may be responsible for the observed wound-healing effect. Saponins have been shown to possess anti-inflammatory effects on the body thereby reducing inflammation during the early phase of the wound, reducing edema, and promoting re-epithelisation of the wound (Kim et al., 2011). They also promote angiogenesis by stimulating the production of vascular endothelial growth factor, through the increase of HIF-1α expression in keratinocytes, and elevation of IL-1β. These result from macrophage accumulation in the wound (Kimura et al., 2006). Tannins are also known to improve wound healing due to their astringent and antimicrobial activities. They also increase epithelisation and the rate of wound contraction and act as free radical scavengers (Okoli et al., 2009). Flavonoids which are known for their antioxidant properties also prevent and slow the onset of cell death and improve vascularity. This improves wound healing by providing nutrients to the affected area. Antioxidants are known to play an important role in wound repair (Okoli et al., 2009). KGE was further shown to be as effective as and a potential alternative wound healing therapy to the positive control, as they were shown to be comparable (p > 0.05; Figure 2b).

Even though KGE demonstrated a dose-dependent effect (Figure 3a), the % contractions from adopted doses were comparable (p > 0.05; Figure 3b). In effect, the smallest dose (1% w/w) was as effective as the highest dose (15% w/w). This may be thought of as welcoming especially when the administration of high doses of plant...
medicines, just as in allopathic medicines, are associated with side and/or adverse effects due to the matrix of compounds in the plant parts adopted (Fatima and Nayeem, 2016).

Conclusions

The outcomes of the study conducted showed that KGE contains some key phytochemicals and these are thought to be responsible for the observed antibacterial, antioxidant, and wound-healing effects. These observations may thus confirm the local uses of *K. grandifoliola* stem bark for the treatment of various wounds, microbial infections, and other ailments associated with oxidative stress in traditional medicine.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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