Amphimas pterocarpoides harms.: An Evaluation of flavonoid and phenolic contents, wound healing, anthelmintic and antioxidant activities of the leaves and stem bark

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HIGHLIGHTS
- Amphimas pterocarpoides leaves and stem bark are used in traditional medicine.
- The leaf and stem bark ointments increased the rate of wound healing in rats.
- The leaf and stem bark methanol extracts caused worm paralysis and death.
- The leaf and stem bark extracts had high phenolic and flavonoid content.
- Remarkable antioxidant activity was shown by the leaf and bark extracts.

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ABSTRACT
The present study evaluated the wound healing, anthelmintic and antioxidant potentials of crude methanol extracts and fractions (petroleum ether, ethyl acetate and methanol) of the leaves and stem bark of Amphimas pterocarpoides. Wound healing activity was determined by the dermal excision model in rats; anthelmintic activity was evaluated by the adult worm motility test using the adult Indian worm, Pheretima postuma. Total flavonoid, phenolic content and antioxidant activity were assessed by the aluminum chloride colorimetric, Folin Ciocalteu, 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging and total antioxidant capacity (TAC) assays respectively. HPLC/UV fingerprints were developed for quality control. The maximum amount of phenolics and flavonoids were detected in the methanol fractions of the stem bark (225.0 ± 20.0 mg/g gallic acid equivalent (GAE) and 201.0 ± 1.41 mg/g quercetin equivalent (QCE) respectively) and leaves (84.54 ± 1.36 mg/g GAE and 130.7 ± 1.71 mg/g QCE, respectively). Both leaf and bark displayed remarkable free radical scavenging and TAC with the highest effect given by the methanol fractions. Significant (p < 0.05) wound contraction was achieved by topical application of the leaf (APL) and stem bark (APS) ointments (5–15%) with >90 % wound surface closure for 1% silver sulphadiazine, APS 15% and APL 10% treated groups by day 15. APL and APS demonstrated a concentration- and time-dependent paralysis and mortality of the P. posthuma with APL (6.25 mg/mL) causing worm paralysis at 82.60 min and death at 93 min, better than 10 mg/mL albendazole (paralysis at 76.30 min; death at 117 min). Tannins, triterpenoids, phytosterols, flavonoids, saponins and coumarins were detected in the leaves and bark. The results have proven the potential of A. pterocarpoides as a wound healing and anthelmintic agent, giving scientific credence to its use in traditional medicine.

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1. Introduction

Leguminosae (also called Fabaceae) is the third largest family in the plant kingdom, comprising of about 700 genera with over 19,000 species of trees, shrubs, lianas and herbs which grow in the terrestrial environments of African countries [1]. Plants of the family Leguminosae are well known for their economic use in timber trade and more emphatically for their valuable use in traditional medicine [2]. Several researchers have investigated a number of Leguminosae plants and reported remarkable biological activities including anti-nociception, anti-inflammatory, anti-oxidant, antimicrobial, antimitic, anti-cancer, anti-parasitic effects, indicating the family as a rich source of diversified natural compounds for drug discovery [1]. A wide variety of plant secondary metabolites with great structural diversity including terpenes, alkaloids, polyphenols, cyanogens, polyketides and peptides have been identified in the family Leguminosae with polyphenols including phenolic acids, flavonoids, coumarins and tannins being the most predominant [3, 4, 5, 6].

The genus Amphimas a small genus of the Leguminosae family, belonging to the subfamily Puplidionaceae and confined to West and Central Africa. Amphimas pterocarpoides is the most common species of the genus widely distributed throughout tropical African countries including Guinea, DR Congo, Sudan, Gabon, Cameroon, Sierra Leone and Ghana [7]. In Ghana the tree is called ‘yaya’ and used in traditional medicine [8]. Traditional medicinal uses of the leaves and bark include treatment of cough, pneumonia, venereal diseases, small pox, measles, chicken pox, swellings, pain, wounds, malaria fever and gouty arthritis [9]. A reddish resin exudate from the bark is used in the treatment of dysentery, anaemia, schistosomiasis, haematuria, dysmenorrhea, mumps, and as antidote to several poisons. The twig is applied to prevent miscarriage and a wood decoction is drunk to treat impotence [7]. In phytochemical studies, isoflavonoids and phenolic acids were identified in the roots and stem bark [10, 11, 12]. In previous bioactivity studies, the bark extracts improved mineral density and strength in the bones of oestrogen deficient rats [13]. Isoflavonoid derivatives including amphisilofavone, 8-methoxyisoformononeton, 6-methoxyisoformonetonin and isoformonetonin from the bark and roots demonstrated antimicrobial, antioxidant and osteogenic activities [14, 15]. The stem bark methanol extract was proven to have a wide safety margin for traditional use [16]. We recently demonstrated that the bark and leaves also possess remarkable anti-inflammatory and antipyretic activities [17]. It is evidenced from literature search that only few scientific reports evaluating the basis of traditional uses of A. pterocarpoides exist. The present study was thus intended to further investigate some biological activities of A. pterocarpoides bark and leaves including wound healing, antihelmintic and antioxidant activities. HPLC-UV fingerprints were also developed for authentication and quality control.

2. Materials and methods

2.1. Harvesting and processing of plant material

Fresh samples of the stem bark and leaves of A. pterocarpoides were harvested from the Crop Research Institute of Ghana, Fumesua, Ghana (−06° 42'51.33"N, 1° 31'44.2"W) in November, 2020. The identity of the samples was confirmed by Dr. George Henry Sam, a botanist at the Herbal Medicine Department, KNUST, Ghana. Herbarium samples with identity codes KNUT/HMI/2018/AP/037 for the leaf and KNUT/HMI/2018/AP/007 for the bark were placed at the Faculty of Pharmacy and Pharmaceutical Sciences Herbarium, KNUST.

2.2. Plant extract preparation

The plant material was rid of all extraneous materials by washing with water, cut into smaller chunks, dried in the sun under a shade for 7 days and crushed into coarse powder with a hammer mill (Lab mill machine, Christy and Norris, Chelmsford, England). For the evaluation of wound healing and antihelmintic activities, 600 g each of the powdered stem bark and leaves were extracted separately with 1000 mL of MeOH by cold maceration for 72 h with occasional stirring. The filtrates obtained were concentrated to dried extracts using a rotary evaporator (Rotavapor BUCHI-200, Hamburg Germany) at 50 °C. A dark green semi solid extract for the leaves and a dark brown powdery extract for the stem bark were obtained. The extracts were referred to as APL and APS for the leaf and bark respectively.

For the assessment of antioxidant activity, total phenolic and total flavonoid contents, three solvent extracts were prepared each for the bark and leaves as follows: 1 kg of the powdered sample was extracted with a Soxhlet apparatus successively with 2L each of petroleum ether (pet-ether), ethyl acetate (EtOAc) and methanol (MeOH) in increasing polarity. The extracts obtained were subsequently referred to as APLp, APLe, APLMe and APSp, APSe, APSMe for the pet-ether, EtOAc and MeOH extracts of the bark and leaves respectively.

2.3. Qualitative phytochemical screening

Preliminary screening of the powdered samples was undertaken to identify the presence of major classes of secondary metabolites including tannins, glycosides, alkaloids, phytosterols following established methods [18].

2.4. Ethical consideration

Ethical approval for the use of experimental animals was obtained from the Animal Ethical Committee (FFPS-AEC/CA01/13), Faculty of Pharmacy and Pharmaceutical Sciences, KNUST. The experimental animals were handled according to the Guidelines for Care and Use of Laboratory Animals (Directive 2010/63/EU; Animal Care and Use Committee, 1998).

2.5. Evaluation of wound healing activity

2.5.1. Experimental animals

Sprague-Dawley rats weighing between 90- 130 g were purchased from the Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana. The animals were housed in the vivarium of the Pharmacology Department, KNUST in steel cages (57 × 34 × 40 cm³) and fed with commercial rat feed, water ad libitum. The following conditions were maintained at the laboratory: temperature: 25 ± 1 °C, relative humidity: 60–70%, 12-h light-dark cycle. These conditions were maintained throughout the days of experimental.

2.5.2. Ointment preparation

The leaf (APL) and stem bark (APS) ointments of concentrations 5, 10 and 15% weighing 25 g each were prepared using a scaled formula for simple ointment according to the British Pharmacopoeia [19] (Table 1). Petroleum jelly was used as the base ointment.

2.5.3. Evaluation of acute dermal toxicity

Following the guidelines of the Organization for Economic Cooperation and Development (OECD) no. 402, the acute dermal toxicity of the formulated ointments was assessed. This was done to ensure that the ointments were not irritant to the animals’ skin at the highest concentrations used. APL (15 %) and APS (15 %) were topically applied on the rats’ bare skin and observed for 14 days [20].

Table 1. Formula for preparing the aqueous cream with the extract.

| Ingredients               | Quantities of ingredients (g) |
|---------------------------|-------------------------------|
| Base (Petroleum Jelly)    | 23.7                          |
| Active ingredient (APL/APS)| 1.25                          |
| Ointment (%)              | 5.00                          |

2
2.6. Antioxidant activity

Where

\[ \text{Radical scavenging activity} = \frac{\text{Abs sample} - \text{Abs control}}{\text{Abs control}} \times 100 \quad [\text{EQ2}] \]

Where ‘Abs sample’ represents the absorbance of test sample/positive control and ‘Abs control’ represents the absorbance of the negative control.

2.6.2. Total antioxidant capacity (TAC)

The antioxidant capacity of the extracts was evaluated following an established method [23]. A line graph [EQ3: \( y = 0.0005850x + 0.07264, \ R^2 = 0.8098 \)] of absorbance versus the respective concentration of gallic acid (3.125–100 \( \mu \)g/mL) was obtained. The TAC was deduced from this standard graph. TAC was expressed as gallic acid equivalent (GAE) in mg/g of dried extract.

2.6.3. Total phenol content (TPC)

The total phenolic content was evaluated by the Folin-Ciocalteu method [24]. Gallic acid (3.125–100 \( \mu \)g/mL) was used as the reference substance. TPC of extracts was deduced from the line equation, \( y = 0.003190x + 0.1358, \ R^2 = 0.9533 \) [EQ4], obtained from a graph of absorbance versus the respective concentration of gallic acid (3.125–100 \( \mu \)g/mL). TPC was expressed as gallic acid equivalent (GAE) in mg/g of dried extract.

2.6.4. Total flavonoid content (TFC)

The total flavonoids was quantified by the aluminum chloride colorimetry method [25]. Quercetin (3.125–100 \( \mu \)g/mL) was used as positive control. A line graph [EQ5: \( y = 0.002382x + 0.05557, \ (R^2 = 0.9920) \)] of absorbance versus the respective concentration of quercetin was obtained and the TFC was deduced from this standard graph. TFC was thus recorded as quercetin equivalent (QCE) in mg/g of dried extract.

2.7. Anthelmintic activity

2.7.1. Collection of adult Perheretima posthuma

The adult Indian earthworm (Phereetima posthuma; Order: Haplotoxida) was used for this assay. This worm was selected because of its availability and more importantly, its physiological and anatomical similarity with Ascaris lumbricoides (intestinal roundworm) [26].

2.7.2. In vitro anthelmintic activity

The anthelmintic activity of the MeOH extracts of the leaves and stem bark of A. pterocarpoides was carried out according to a previously described method [27]. The anthelmintic activity of plant extracts was studied by evaluating their effect on worms after direct exposure for a period of time. Different working concentrations of the MeOH extracts was prepared by serial dilution with normal saline to obtain concentrations between 50 and 1.56 mg/mL. The reference drug used was albendazole (10 mg/ml) and normal saline served as the negative control. Briefly, four earthworms each were placed in separate petri dishes filled with 30ml of the extract/normal saline/albendazole. The worms were observed for their natural movements as well as evoked responses. The time taken for the worms to be paralyzed and/or die was recorded. Paralysis was recorded when no movements of any sort (wiggling movement) could be observed except when the worm was shaken vigorously or pricked with a pin. Death of worm was recorded when worms failed to move after being shaken vigorously or when immersed in warm water (50 °C) followed by fading of their skin colour. The results were expressed in comparison to the standard drug albendazole. Concentrations of extracts that produced activity after 200 min were considered inactive.

2.8. HPLC fingerprinting

The HPLC fingerprinting of the MeOH extracts (1 mg/mL) was performed for authentication and quality control. The system consisted of a PerkinElmer Flexar HPLC attached to a PDA detector. Separation was achieved on Agilent Zorbax 300SB, C18 (250 × 4.6mm, 5um) column. The mobile phase consisted of 0.05% Trifluoroacetic acid (A) and methanol (B). A simple linear gradient program beginning with 90% A to 10% B for 3 min, followed by an isocratic step of 90% B for 25 min then a return to the initial 90% A to 10% A for 5 min for re-equilibration of the system between individual runs was used. An injection volume of 20\( \mu \)L, flow rate of 1 ml/min, and a wavelength of 275nm were employed.

2.9. Data analysis

Results were recorded as the mean ± standard error of the mean (SEM). Significant differences between the treatment groups and the negative control were determined by the One-Way Analysis of Variance (ANOVA) followed by Dunnet’s Multiple Comparisons Test. \( P \leq 0.05 \) was considered statistically significant. GraphPad for Windows version 6 (GraphPad Prism Software, San Diego, USA) was used for the analysis.
3. Results

3.1. Phytochemical analysis

Results of the qualitative phytochemical analysis of the powdered leaf and bark of *A. pterocarpoides* are presented on Table 2.

3.2. Wound healing activity

The highest dose of the leaf and bark ointments of *A. pterocarpoides* caused no skin irritation, physical changes or any form of toxic effect to the skin in acute dermal toxicity studies.

Significant (*p* < 0.05) decrease in wound surface area was observed for all extracts (APL and APS) and 1% silver sulphadiazine (SSD)-treated groups compared to the negative control group. Figures 1A and 1B show the gradual reduction in the wound surface diameter estimated every other day beginning from the 3rd day after wound creation. Table 3 records the percentage wound surface closures calculated for all treatment and negative control groups from day 3–21. The stem bark ointment (APS) showed a concentration-dependent wound healing activity while the effect of the leaf ointment (APL) was not concentration-dependent. The overall wound healing potential for test samples was as follows: SSD > APS15% > APS 10% = APL 5% > APL10% > APS 5% = APS 15%. For all extract treated groups, remarkable wound healing (~70% wound surface closure) occurred from day 12–21 whereas the positive control (SSD 1%) exhibited more than 80% wound contraction by day 9 post wound excision. By day 15, significant (*p* < 0.005) wound healing (>90% wound closure) was observed for 1% SSD, APS 15% and APL 10% treated groups (Figure 1A, Table 3). During the experiment, the rate of wound contraction in the negative control group was remarkably lesser than that of the extracts and SSD-treated groups.

3.3. Antioxidant activity

3.3.1. DPPH free radical scavenging activity

The DPPH free radical scavenging activity of the various solvent extracts of the leaf and stem bark of *A. pterocarpoides* was expressed as IC₅₀ obtained from non-linear regression curves as shown in Figure 2. From the results, both bark and leaf extracts displayed remarkable free radical scavenging effect which increased with increasing concentration. In general, the stem bark extracts (IC₅₀ range from 15.38 to 199.20 μg/mL) had a higher radical scavenging effect than the leaf extracts (IC₅₀ range from 23.30 to 278.4 μg/mL). The MeOH extracts of the bark and leaves had the highest radical scavenging effect with IC₅₀ of 15.38 and 23.30 μg/mL respectively comparable to that of gallic acid (IC₅₀ = 15.62 μg/mL). The radical scavenging effect of test extracts was as follows: APS₅₀ > Gallic acid > APL₅₀ > APS₁₀ > APL₁₀ > APS₁₅ > APS₂₀ > APL₂₀. The IC₅₀ of all samples including the positive control are presented on Table 4.

3.3.2. Total antioxidant capacity (TAC)

The total antioxidant capacity was determined from a standard line graph [EQ3] developed using gallic acid (Figure 3A) and was thus recorded as gallic acid equivalent (GAE). Significant variations were observed among the TAC of the leaf and bark extracts which also varied according to the type of solvent extract (Table 4). For both leaf and bark, TAC increased in the order APS₅₀ > APL₅₀ > APS₁₀ > APL₁₀ > APS₁₅ > APS₂₀ > APL₂₀ (Figure 3B). The MeOH extracts had the highest TAC. Generally, the stem bark extracts had a higher TAC than the leaf extracts. Comparing the TAC of the various solvent extracts, it was observed that no significant difference existed between the TAC of the MeOH extracts of the leaves and stem bark (APS₅₀ = 126.0 ± 1.06; APL₅₀ = 124.6 ± 1.00 mg/g GAE). However, the EtOAc extracts of the bark and leaves had higher TAC than the pet-ether extracts (Table 4, Figure 3B).

3.3.3. Total phenolic content (TPC)

The total phenolic content in the leaves and stem bark of *A. pterocarpoides* was determined from a standard curve of gallic acid [EQ4] (Figure 3C) and was expressed as gallic acid equivalent (GAE). The total phenolic content varied between the two plant parts and also with respect to the solvent used for extraction. The stem bark had a higher phenolic content (52.7–225.0 mg/g GAE) than the leaves (45.91–84.54 mg/g GAE). Over all the MeOH extracts of the stem bark (225.0 ± 20.08 mg/g GAE) and leaves (84.54 ± 1.36 mg/g GAE) recovered the highest amount of phenolic content compared to other solvent extracts (Figure 3D). TPC decreased with decreasing polarity of solvent hence the pet-ether fraction has the least TPC. In the leaf extract there was no significant difference between the TPC of the EtOAc and pet-ether extracts (Table 4).

3.3.4. Total flavonoid content (TFC)

The total flavonoid content was determined from the standard curve of quercetin [EQ5] (Figure 3E) and was expressed as quercetin equivalent (QCE). The TFC for the leaf extracts ranged from 53.19 to 130.7 mg/g QCE and from 82.50 to 201.0 mg/g QCE for the stem bark (Table 4). Among the three solvents used, methanol extracted the maximum amounts of flavonoids, followed by ethyl acetate. Pet-ether was the least effective in extracting flavonoids from both leaves and stem bark. The stem bark had a higher flavonoid content (201.0 ± 1.41 mg/g QCE) than the leaf extract (130.7 ± 1.71 mg/g QCE). There was no significant difference between the TFC of EtOAc and pet-ether fractions of the leaf (Table 4; Figure 3F).

3.4. Anthelmintic activity

The anthelmintic activity of the crude MeOH leaf (APL) and the stem bark (APS) extracts were recorded as paralysis time and death time in minutes. The results are presented on Table 5.

3.5. HPLC fingerprinting

The chemical fingerprints of the MeOH leaf (APL) and stem bark (APS) extracts (1 mg/mL) were determined for the purpose of authentication and quality control (Figure 4). Due to potential peak shifting, which could arise from variations in chromatographic conditions, the retention times were converted to relative retention times. Briefly, one prominent peak was selected as the reference peak to calculate the relative retention times of other peaks in each chromatogram. From the results, ten prominent peaks were identified in the leaf extract and eight in the stem bark extract (Table 6). By comparison of their relative retention times, seven peaks were common to both leaf and stem bark extracts. For all peaks (except peak 10), higher intensities were observed in the leaf extract than in the stem bark extract indicating relatively higher amounts of the individual components in the leaf.

Table 2. Phytochemical screening of leaf and stem bark of *A. pterocarpoides*.

| Constituent       | Test          | Leaf          | Stem bark     |
|-------------------|---------------|---------------|---------------|
| Tannins           | Ferric chloride test | +            | +             |
| Flavonoids        | Alkaline reagent test | +            | +             |
| Alkaloids         | Dragendorff's test | -            | +             |
| Coumarins         | Fluorescence test | +            | +             |
| Glycosides (general) | Fehling's test     | +            | +             |
| Saponins          | Frothing test   | +            | +             |
| Anthraquinones    | Borntrager's test | -            | -             |
| Triterpenoids     | Salkowski's test | +            | +             |
| Phytosterols      | Liebermann Buchard's test | +          | +             |

(+) Detected; (-) Not Detected.
A. pterocarpoides bark extract, APSP-MeOH leaf extract, APS-
marker compounds in extracts [28, 29]. For most herbal extracts
techniques such as HPLC-UV and HPLC-MS to identify and quantify
most authors have utilized chromatography coupled with spectral
detected only in the bark. For the quality control of herbal extracts,
tosterols, tannins and coumarins in both plant parts. Alkaloids were
bark showed the presence of saponins, triterpenoids,
ng for quality control.

tiferulic activity, phenolic and
of A. pterocarpoides of
avonoid contents of various solvent fractions
ngrimetry of the extracts were also
ded and can thus not
ngrimetry where
the chromatographic pattern of chemically characteristic constituents in
the extract is developed [30]. By the concept of phytoequivalence, the
chromatographic pattern of chemically characteristic constituents in
ed by a process called chemical
ngprints of the extracts were also
ded for the MeOH extracts of A. pterocarpoides leaves and stem
avonoids, phy-
thence, the complexity of mixtures of phytoconstituents makes identi-
fying potential marker compounds usually difficult. Sometimes, the
active principles have not been conclusively identified and can thus not
accurately quantified. Some studies have recommended the evalua-
cultural factors that affect phytochemicals, and can thus be used for
ng and quality control of specific species [31]. In this study, HPLC
anidates have been developed for the MeOH extracts of A. pterocarpoides leaves and stem
bark. Together with other parameters [17], these chromatograms can be
used for authentication and quality assessment of A. pterocarpoides crude
samples and herbal preparations.

4. Discussion

The present investigation describes the wound healing and anthel-
mimic activity of the crude methanol extracts of the stem bark and leaves of A. pterocarpoides as well as a comparative evaluation of the antioxidant activity, phenolic and flavonoid contents of various solvent fractions from both plant parts. HPLC fingerprints of the extracts were also developed for quality control.

Qualitative phytochemical screening of the powdered leaves and bark showed the presence of saponins, triterpenoids, flavonoids, phytosterols, tannins and coumarins in both plant parts. Alkaloids were detected only in the bark. For the quality control of herbal extracts, several authors have utilized chromatography coupled with spectral techniques such as HPLC-UV and HPLC-MS to identify and quantify marker compounds in extracts [28, 29]. For most herbal extracts

Table 3. Wound healing activity of A. pterocarpoides leaf and stem bark extracts.

| Treatment Group | Percentage (%) wound closure |
|-----------------|-------------------------------|
|                 | Day 3 | Day 6 | Day 9 | Day 12 | Day 15 | Day 18 | Day 21 |
| APL 5           | 26.90 ± 6.90 | 55.23 ± 4.41 | 64.45 ± 9.54 | 81.30 ± 5.86 | 81.96 ± 7.55 | 88.67 ± 5.05 | 99.06 ± 4.17 |
| APL 10          | 5.89 ± 1.09 | 15.64 ± 4.74 | 65.93 ± 5.47 | 90.03 ± 2.01 | 91.56 ± 3.85 | 93.67 ± 3.37 | 99.68 ± 6.16 |
| APL 15          | 5.43 ± 0.57 | 10.07 ± 4.70 | 55.17 ± 9.75 | 72.59 ± 13.49 | 83.90 ± 12.15 | 89.19 ± 8.34 | 95.92 ± 5.88 |
| APS 5           | 16.39 ± 2.63 | 39.36 ± 4.11 | 62.10 ± 6.61 | 75.17 ± 6.85 | 78.32 ± 5.86 | 85.32 ± 12.1 | 95.45 ± 6.79 |
| APS 10          | 10.14 ± 1.14 | 27.51 ± 3.38 | 71.13 ± 6.31 | 79.78 ± 4.39 | 89.56 ± 2.83 | 94.56 ± 6.15 | 99.59 ± 6.06 |
| APS 15          | 18.87 ± 2.97 | 52.39 ± 2.85 | 64.44 ± 6.58 | 83.93 ± 6.81 | 90.95 ± 7.85 | 94.68 ± 7.77 | 100.00 |
| SSD 1%          | 14.62 ± 1.63 | 47.39 ± 2.01 | 80.91 ± 6.77 | 85.91 ± 5.46 | 97.47 ± 10.59 | 100.00 | 100.00a |
| NS              | 0.61 ± 0.06 | 22.52 ± 3.03 | 41.06 ± 2.64 | 62.45 ± 1.60 | 67.37 ± 2.89 | 76.11 ± 6.77 | 79.82 ± 6.94 |

APL: A. pterocarpoides MeOH leaf extract; APS: A. pterocarpoides MeOH stem bark extract; NS: normal saline; SSD: silver sulphadiazine; [a: p < 0.005; b: p < 0.01; c: p < 0.05- compared to the negative control].

Figure 1. Wound surface area (mm²) measured over 21 days of treatment with MeOH extracts of the leaves (A) and stem bark (B) of A. pterocarpoides [APL- A. pterocarpoides MeOH leaf extract, APS- A. pterocarpoides MeOH stem bark extract, SSD-silver sulphadiazine 1%, NS-normal saline].

Figure 2. DPPH free radical scavenging of various extracts from the leaves (A) and stem bark (B) of A. pterocarpoides [APLM- A. pterocarpoides MeOH leaf extract, APLe- A. pterocarpoides EtOAc leaf extract; APLp- A. pterocarpoides pet-ether leaf extract; APSM- A. pterocarpoides MeOH stem bark extract; APSp- A. pterocarpoides EtOH stem bark extract, APSp- A. pterocarpoides pet-ether stem bark extract, GA-gallic acid].

However, the complexity of mixtures of phytoconstituents makes identifying potential marker compounds usually difficult. Sometimes, the active principles have not been conclusively identified and can thus not be accurately quantified. Some studies have recommended the evaluation of whole extracts by a process called chemical fingerprinting where the chromatographic pattern of chemically characteristic constituents in the extract is developed [30]. By the concept of phytoequivalence, the chromatographic fingerprint of a plant extract measured under the same conditions will consistently demonstrate the exact similarity and/or differences and can thus be used for authentication and quality control of specific species [31]. In this study, HPLC fingerprints have been developed for the MeOH extracts of A. pterocarpoides leaves and stem bark. Together with other parameters [17], these chromatograms can be used for authentication and quality assessment of A. pterocarpoides crude samples and herbal preparations.
The methanol extracts of the bark and leaves of *A. pterocarpoides* displayed significant increase in the rate of wound contraction, indicating a clear response to treatment unlike the negative control group. Plant secondary metabolites such as tannins, flavonoids and triterpenoids are known to positively influence wound healing [32]. The MeOH extracts were also found to contain high amounts of phenolic compounds and flavonoids. Tchoumtchoua et al., (2013) identified several isoflavonoid derivatives in the stem bark of *A. pterocarpoides* [10]. Flavonoids are known to significantly influence wound healing by their anti-inflammation, antioxidant and angiogenesis properties which promote wound contraction, capillary regeneration, collagen deposition and re-epithelialization [33]. Tannins are able to stimulate dermal fibroblasts and keratinocytes, resulting in an increased secretion of collagen and regeneration of epithelial cells [34]. Triterpenes reduce inflammation and the generation of reactive oxygen species in the wound microenvironment, expediting the tissue repair process [35, 36]. These phytoconstituents also have antimicrobial and astringent properties which promote wound healing as well as improve the rate of epithelialization [37]. In our previous study, the stem bark and leaves of *A. pterocarpoides* demonstrated remarkable anti-inflammatory activity which supports the observed wound healing effect [17]. The wound healing activity of *A. pterocarpoides* could thus be attributed to the individual or combined effect of the constituents in the plant.

The role of antioxidants in enhancing and accelerating wound healing cannot be underestimated [38]. Among plant secondary metabolites, phenolic compounds such as phenolic acids, tannins, flavonoids and coumarins have shown promising antioxidant effects in several models [32]. This study has revealed that the MeOH extracts of the bark and leaves contained the highest amounts of phenolic constituents and flavonoids compared to the EtOAc and pet-ether fractions. The results was congruent with previous studies which also reported MeOH as the best solvent for extracting phenolic compounds [39, 40]. Tchoumtchoua et al., (2013) recommended the use of methanol as solvent for extracting

| Table 4. Antioxidant activity of the leaf and stem bark of *A. pterocarpoides*. |
|---------------------------------|--------|--------|--------|--------|
|                                | DPPH (μg/g mL) | TAC (mg/g GAE) | TPC (mg/g GAE) | TFC (mg/g GCE) |
| APLM 23.30                     | 124.6 ± 1.00 | 84.54 ± 1.36 | 130.7 ± 1.71 |
| APLP 278.4                     | 67.12 ± 0.63 | 48.47 ± 1.27 | 54.44 ± 2.37 |
| APSM 15.38                     | 260.0 ± 1.06 | 225.0 ± 20.0 | 201.0 ± 1.41 |
| APSF 56.42                     | 105.0 ± 7.02 | 82.0 ± 7.07 | 157.0 ± 2.89 |
| ApSel 199.20                   | 86.0 ± 1.41  | 52.7 ± 0.66  | 82.50 ± 3.56  |

Gallic acid 15.62

A. *pterocarpoides* MeOH leaf extract; APLM: A. *pterocarpoides* EtOAc leaf extract; APLP: A. *pterocarpoides* pet-ether leaf extract; APSM: A. *pterocarpoides* MeOH stem bark extract; APSF: A. *pterocarpoides* EtOH stem bark extract, APSE: A. *pterocarpoides* pet-ether stem bark extract, GAE-gallic acid equivalent, QCE- quercetin equivalent.

**Figure 3.** Total antioxidant capacity (A, B), total phenolic content (C, D) and total flavonoid content (E, F) of the leaves and stem bark of the MeOH, EtOAc and pet-ether extracts of *A. pterocarpoides*.
maximum amount of phenolic content from *A. pterocarpoide* [10]. Polar solvents are the most suitable for maximum extraction of phenolic compounds due to the chemical nature of the latter. Remarkable DPPH radical scavenging and total antioxidant capacity was also exhibited by the MeOH fractions of both stem bark and leaf. In a previous study, the MeOH–CH2Cl2 (1:1) extract of *A. petrocarpoide* roots exhibited DPPH radical scavenging effect (IC50 = 63.59) [14]. Previous works have demonstrated a positive correlation between phenolic content, antioxidant activity and wound healing effects of plant extracts [41, 42]. This suggests that the amount of phenolic content in *A. pterocarpoide* greatly influences its antioxidant activity and in turn its wound healing activity.

The stem bark of *A. pterocarpoide* is used in the treatment of schistosomiasis in traditional medicine, giving an indication of possible effect on helminth parasites. The stem bark and leaf MeOH extracts

### Table 5. Anthelmintic activity of the leaf and stem bark of *A. pterocarpoide*.

| Treatment | Concentration (mg/mL) | Time of paralysis (min) (Mean ± SEM) | Time of death (min) (Mean ± SEM) |
|-----------|------------------------|--------------------------------------|----------------------------------|
| Normal saline | -                      | NP                                   | ND                               |
| Albendazole | 10                    | 76.30 ± 0.721                        | 117.4 ± 1.209                   |
|            | 25                    | 43.80 ± 1.212                        | 54.09 ± 1.670                   |
|            | 12.5                  | 59.73 ± 1.289                        | 70.30 ± 0.600                   |
|            | 6.25                  | 82.60 ± 0.300                        | 93.27 ± 0.723                   |
|            | 3.12                  | 110.57 ± 0.208                       | 151.10 ± 3.538                  |
|            | 1.56                  | 163.80 ± 0.264                       | 200.73 ± 1.331                  |
| APL        | 50                    | 27.23 ± 0.711                        | 54.87 ± 1.500                   |
|            | 25                    | 47.97 ± 1.900                        | 69.87 ± 1.650                   |
|            | 12.5                  | 75.70 ± 1.450                        | 93.27 ± 1.193                   |
|            | 6.25                  | 180.04 ± 1.264                       | 130.03 ± 8.328                  |
|            | 3.12                  | 130.00 ± 0.702                       | 175.50 ± 0.360                  |
|            | 1.56                  | 163.23 ± 0.305                       | ND                               |

APL: *A. pterocarpoide* MeOH leaf extract; APS: *A. pterocarpoide* MeOH stem bark extract; NP: no paralysis; ND: no death.

### Table 6. Retention and relative retention times for major peaks in the MeOH extracts of the leaf and stem bark of *A. pterocarpoide*.

| Peak | APL | APS |
|------|-----|-----|
|      | Retention time (min) | Relative retention time (min) | Retention time (min) | Relative retention time (min) |
| 1.   | 13.46 | 1.00 | 13.55 | 1.00 |
| 2.   | -     | -    | 16.41 | 0.83 |
| 3.   | 16.88 | 0.80 | 16.66 | 0.80 |
| 4.   | 17.68 | 0.77 | 17.58 | 0.77 |
| 5.   | 17.83 | 0.75 | 18.08 | 0.75 |
| 6.   | 18.65 | 0.72 | -     | -    |
| 7.   | 18.86 | 0.71 | -     | -    |
| 8.   | 19.19 | 0.70 | 19.26 | 0.70 |
| 9.   | 19.58 | 0.68 | 19.93 | 0.68 |
| 10.  | 20.25 | 0.66 | 20.25 | 0.66 |
| 11.  | 23.47 | 0.57 | -     | -    |

APL: *A. pterocarpoide* MeOH leaf extract; APS: *A. pterocarpoide* MeOH stem bark extract.

Figure 4. HPLC chromatograms of the MeOH extracts of the leaves (A) and stem bark (B) of *A. pterocarpoide* (measured at 275 nm).
demonstrated a concentration- and time-dependent paralysis and mortality of the adult earth worm, *P. posthuma*. Overall, the leaf extract showed a better anthelmintic effect than the stem bark extract. At an exposure concentration of 6.25 mg/mL, APL caused worm paralysis at 82.60 min almost comparable to the effect of the standard anthelmintic avermectin at a concentration of 6.25 mg/mL, APL caused worm paralysis at 82.60 min almost comparable to the effect of the standard anthelmintic avermectin at 82.60 min. Interestingly, APL (6.25 mg/mL) caused death of worms at the 93rd minute better than albendazole (10 mg/mL) which caused death at 117th minute. The anthelmintic effect of medicinal plants has been attributed to constituents such as terpenoids, alkaloids and polyphenols particularly tannins [43, 44]. Tannins, have been shown to possess significant anthelmintic activity against a wide range of nematodes through interactions with proline-rich proteins on the worm cuticle or gastrointestinal tract interfering with worm motility, feeding and absorption of nutrients in the intestines [45]. Saponins are generally known to interact with cell membrane causing changes in membrane permeability resulting in possible alteration of some biological functions [26, 46]. Alkaloids may affect the central nervous system resulting in paralysis and death of worms [43]. The occurrence of these phytochemicals in the leaves and bark of *A. pterocarpoides* may influence its anthelmintic effect.

The current results provide first-hand information on some bio-activities of the bark and leaves of *A. pterocarpoides*. Further investigations on the exact mechanisms of action as well as phytochemical analysis employing chromatography and spectrophotometric methods such as mass spectrometry to identify specific compounds responsible for the observed bioactivities are imperative and underway.

5. Conclusion

This study has provided scientific evidence that topical application of *A. pterocarpoides* leaves and stem bark ointments on skin lesions accelerates wound healing. The MeOH extracts of the leaf and stem bark also possess remarkable anthelmintic and antioxidant activity attributable to appreciable amounts of phenolic and flavonoid content. HPLC chromatograms developed for the leaves and stem bark can serve as fingerprints for the quality assessment of preparations containing *A. pterocarpoides* leaf and stem bark. The presence tannins, flavonoids, saponins, coumarins, alkaloids and triterpenoids in both plant parts may be responsible for the observed therapeutic effects of the plant in traditional medicine.

Declarations

Author contribution statement

Evelyn Asante-Kwataia: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Silas Adjei: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Yakubu Jibira: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Lord Gyimah: Performed the experiments; Analyzed and interpreted the data.

George Adjei-Hinne: Contributed reagents, materials, analysis tools or data.

Isaac Kingsley Amplonshah: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Abraham Yeboba Mensah: Conceived and designed the experiments.

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Data availability statement

Data included in article supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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References

[1] J.K. Benjamin, K.A. da Costa, A.S. Santos, Chemical, botanical and pharmacological aspects of the Leguminosae, Phcog. Rev. 14 (28) (2021) 106–120.
[2] S. Catarino, M.C. Duarte, E. Costa, P.G. Carrero, M.M. Romeiras, Conservation and sustainable use of the medicinal Leguminosae plants from Angola, Peer J 7 (2019), e6736.
[3] H. Singh, P. Chahal, A. Mishra, A.K. Mishra, An up-to-date review on chemistry and biological activities of Senna occidentalis (L.) Link Family: Leguminosae, Adv. Trad. Med. 20 (3) (2020) 263–278.
[4] N.C. Veitch, Isoflavonoids of the Leguminosae, Nat. Prod. Rep. 24 (2) (2007) 417–464.
[5] Y.L. Chew, E.W. Chan, P.L. Tan, Y.Y. Lim, J. Stanslas, J.K. Goh, Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of Leguminosae medicinal plants in Peninsular Malaysia, BMC Compl. Alternative Med. 11 (1) (2011) 1–10.
[6] M.A. Lalacille-Dubois, D.E. Pegnymb, O.P. Noté, A.C. Maitaine-Offer, A review of acaricidal-type saponins from Leguminosae-Mimosoideae as potent cytotoxic and apoptosis inducing agents, Phytochemistry Rev. 10 (4) (2011) 565–584.
[7] A. Tchinda, P. Tane, Amphimias pterocarpoides harms, in: Plant Resources of Tropical Africa Timber 1, PROTA Foundation, Wageningen/Backhuys Publishers Leiden/CTA, Wageningen, 2008, pp. 72–75.
[8] J. Ayazkwa, Strength properties of yaya (Amphimias pterocarpoides), Ghana J. For. 1 (1994) 57–59.
[9] H.M. Burkill, The Useful Plants of West Tropical Africa, ume 2, Royal Botanic Gardens, 1994. Families E-L.
[10] J. Tchoumtchoua, D. Njamen, J.C. Mbanya, A.L. Sklabsounis, M. Halabalaki, Structure oriented UHPLC-LTQ Orbitrap-based approach as a dereplication strategy for the identification of isoavonoids from Amphimias pterocarpoides crude extract, J. Mass Spectrom. 48 (5) (2013) 561–575.
[11] C.W. Bevan, D.E. Ekong, M.E. Obani, J.W. Powell, West African timbers. Part XIII. Extracts from the heartwood of Amphimias pterocarpoides and Pterocarpus erinaceus, J. Chem. Soc. C. Org. (1966) 509–510.
[12] J. Tchoumtchoua, M. Halabalaki, D. Njamen, J. Mbanya, L. Sklabsounis, Isolation of isoavonoids from Amphimias pterocarpoides and structure elucidation using LC-HRMSn and NMR techniques, Planta Med. 76 (12) (2010) P229.
[13] A. Patnak, J. Tchoumtchoua, C. Passali, P. Lebos, S. Kourkoulis, The protective effect of Amphimias pterocarpoides plant extract on bone mineral density and strength in estrogen deficient ovariectomized Wistar rats, Med. Aromatic Plants 5 (5) (2016) 1–7.
[14] E.P. Saah, V.T. Sietinou, V. Kuete, S.T. Lacmata, A.E. Nkengfack, Antimicrobial and antioxidiant isoavonoid derivatives from the roots of *Amphimias pterocarpoides*, Z. Naturforchem. B. Chem. Sci. 68 (8) (2013) 931–958.
[15] J. Tchoumtchoua, M. Makropoulou, S.B. Ateba, A. Boulaka, M. Halabalaki, J.C. Mbanya, A.L. Sklbsounis, D.J. Mitsiou, Estrogenic activity of isoavonoids from the stem bark of the tropical tree *Amphimias pterocarpoides*, a source of traditional medicines, J. Steroid Biochem. Mol. Biol. 158 (2016) 138–148.
[16] J. Tchoumtchoua, O.R. Mouchili, S.B. Ateba, S. Zingue, M. Halabalaki, J.C. Mbanya, A.L. Sklbsounis, D. Njamen, Safety assessment of the methanol extract of the stem bark of Amphimias pterocarpoides harms: acute and subchronic oral toxicity studies in Wistar rats, Toxicol. Rep. 1 (2014) 877–884.
[17] G. Adjei-Hinne, G. Komлага, E. Asante-Kwataia, A.Y. Mensah, Quality control standardization and evaluation of the anti-inflammatory and antipyretic effects of the leaves and stem bark of *Amphimias pterocarpoides* harms (Leguminosae), J. Pharmacogn. Phytotherapy 13 (2) (2021) 46–59.
[18] W.C. Evans, Trease and Evans’ Pharmacognosy E-Book, Elsevier Health Sciences, 2009.
[19] British Pharmacopoeia, Her Majesty Stationary Office, 2, 2009. London.
[20] D.D. Kokane, R.Y. More, M.B. Kale, M.N. Nehete, P.C. Mehandale, C.H. Gadgoli, Evaluation of wound healing activity of root of *Mimosa pudica*, J. Ethnopharmacol. 124 (2) (2009) Jul 15) 311–315.
