Full Length Research Paper

Quality control standardization and evaluation of the anti-inflammatory and antipyretic effects of the leaves and stem bark of *Amphimas pterocarpoides* harms (Leguminosae)

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*Amphimas pterocarpoides* is used traditionally for the treatment of oedema, infections, malaria and skin diseases. This study described pharmacognostic and physicochemical parameters of the leaf and stem bark. The carrageenan-induced foot oedema and the baker’s yeast-induced pyrexia were used to assess the anti-inflammatory and antipyretic activities respectively. The leaf of *A. pterocarpoides* is oddly pinnate compound, oblong in shape with acuminate apex and obtuse base. The stem bark is scaly and rugged and exudes a reddish resin when bruised. Leaf microscopy displayed wavy-walled epidermal cells, paracytic stomata, glandular and clothing trichomes, bean-shaped lignified vascular bundle, prismatic calcium oxalate crystals. The leaf (APL) and stem bark (APSB) extracts (30-300 mg/kg p.o.) significantly inhibited foot oedema in chicks with maximum percentage inhibition of 76.65 ± 6.27 % and 77.04 ± 2.53% respectively [ED₅₀ = 225.3 ± 31.52 mg/kg (APL) and 178.9 ± 29.57 mg/kg (APSB)]. APL, APSB and paracetamol also showed significant antipyretic activity [ED₅₀ = 70.95 ± 9.24 mg/kg (APL), 27.02 ± 1.96 mg/kg (APSB), 32.47 ± 3.03 mg/kg (Paracetamol)]. Tannins, flavonoids, phytosterols, triterpenoids and coumarins were identified in *A. pterocarpoides*. This study has provided essential diagnostic characteristics for the quality control of *A. pterocarpoides* and justified its anti-inflammatory and antipyretic effects.

**Key words:** Pharmacognosy, carrageenan, *Amphimas pterocarpoides*, pyrexia, inflammation.

INTRODUCTION

*Amphimas pterocarpoides* is a deciduous forest tree known for numerous medicinal uses in Africa. The leaves and bark are used for the treatment of respiratory and urinary tract infections, pain, sexually transmitted...
infections, skin rashes such as measles, small pox and chicken pox, oedema, malaria fever, sores and gout (Ayarkwa, 1994). The stem bark resin is also used to treat dysentery, anemia, haematouria, dysmenorrhea, blennorrhoea, mumps, schistosomiasis and as an antidote to several poisons. A decoction of the inner bark and twigs is used to treat cough and as a steam bath against impotence and to prevent miscarriage (Tchinda and Tané, 2008).

In the rural parts of Ghana where herbal medicine often represents the main therapeutic system of healthcare, adulteration/substitution of plant materials with inferior substances is a major challenge (WHO, 2007). In parts of Africa for example where several species of *Amphimas* occur, the wood of *A. pterocarpoides* being very similar to that of *A. ferrugineus* are often mixed during trade. *Milicia excelsa* wood is also considered a suitable substitute for the wood *A. pterocarpoides* (Tchinda and Tané, 2008). This trend is unacceptable, dangerous and can lead to un-reproducible therapeutic effect when using the plant material for medicinal purposes. In facing this challenge, this study was set out to provide useful diagnostic features for the correct identification and quality control of *A. pterocarpoides*. If correctly applied, these parameters will ensure the efficacy and safety of herbal products prepared from the plant and in the end contribute to protecting and improving the health and wellbeing of patrons (Dangar and Patel, 2018; Chanda, 2014).

* A. *pterocarpoides* has been reported for protective effect on bone mineral density and strength (Patsaki et al., 2016). Isoflavonoids from the roots and stem also showed antimicrobial, antioxidant (Saah et al., 2013) and oestrogenic activity (Tchoumtchoua et al., 2016). The current study was undertaken to evaluate the anti-inflammatory and antipyretic activity of the leaves and stem bark which are traditionally used for this purpose and compare the activities of these parts to provide knowledge on the most effective part of the plant.

**MATERIALS AND METHODS**

**Chemicals and drugs**

The following drugs and chemicals were used: carrageenan (Sigma-Aldrich, Inc., St. Louis, MO, USA), diclofenac and dexamethasone (Ernest Chemist, Ghana), paracetamol (PhytoRiker, Accra, Ghana), commercially available dried baker’s yeast (*Saccharomyces cerevisiae*, Saf do Brasil Produtos Alimenticios Ltd, Brazil).

**Animals**

Day-old chicks (*Gallus gallus*; strain; Shaver 579, one-day post hatch) were purchased from Akate farms, Kumasi, Ghana. The chicks were housed in stainless steel cages (34×57×40 cm) in groups of 12 to 13 per cage and kept in the Animal House in the Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Ghana. Room temperature was maintained at 29°C with an overhead incandescent lamp. During the week of acclimatization, the animals were fed with chick mash (GAFCO, Tema, Ghana) and provided with drinking water *ad libitum*. A week old chicks were used for the bioassay.

Sprague-Dawley rats (100-200 g) were purchased from the Noguchi Memorial Institute of Medical Research (NMIMR), University of Ghana, Accra and housed at the Pharmacology Department, KNUST for acclimatization until use. The animals were housed in groups of 5-6 in stainless steel cages (34×47×18 cm) with soft wood shaving as bedding, fed on local commercial chow and given water *ad libitum*. Conditions of the laboratory were maintained at 24-28°C, relative humidity 60-70% and 12 h light-dark cycle. All experimental procedures were in accordance with the principles for the care and use of experimental animals (Directive 2010/63/EU). Ethical approval was also given by the Departmental Ethics Committee.

**Plant collection and extraction procedure**

Leaves and stem bark of *A. pterocarpoides* were collected from the premises of the Crop Research Institute of Ghana, Fumesua, Ghana [-06°42'51.3''N, 1° 31'44.2''W] in November, 2018. The materials were identified and authenticated by Mr. Jonathan Dabo, a Senior Field Technician at Forestry Research Institute of Ghana (FORIG), Ghana. The materials were washed with water to remove all unwanted materials. Sample specimens with voucher numbers KNUST/HM1/2018/AP/037 and KNUST/HM1/2018/AP/007 for the leaves and stem bark respectively were deposited at the herbarium of the Department of Herbal Medicine in the Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana. The fresh leaves and stem bark of *A. pterocarpoides* were shade-dried for a week and milled into coarse powder using an electric mill. One hundred grams of powdered material was cold macerated with 1 L of methanol for 72 h with occasional shaking. The extract was filtered and concentrated on a rotary evaporator to obtain the crude extracts of yields 15.74 and 6.57% for the leaves and stem bark respectively.

**Pharmacognostic studies**

**Organoleptic and macro-morphological studies**

The whole leaf and the slashed stem bark were examined for sensory and macro-morphological features. These included colour, odour, texture and taste of the fresh samples. Leaf lamina surface characteristics such as leaf base, shape, venation, apex and margin type were also noted, fifty fresh leaves were randomly selected and used in the determination of the average length and width of the leaf. The fresh stem bark was investigated for surface characteristics, curvature and fracture type (Baidoo et al., 2019).

**Qualitative microscopy of the fresh leaf**

Thin handmade sections of the fresh leaf lamina and transverse sections (T/S) of the midrib and petiole of the fresh leaf were made using sharp razor blades. The sections were cleared by boiling in 80% chloral hydrate solution for an hour until the green pigment was lost. The sections were then stained with aqueous solution of 1% haematoxylin and a drop of 0.1% phloroglucinol plus a drop of concentrated hydrochloric (HCl). Photomicrographs were taken at magnifications ×40 and ×10 (Komiaga et al., 2014).
**Quantitative microscopy of fresh leaf**

Quantitative leaf parameters including palisade ratio, stomatal number, stomatal index, vein – islet and veinlet termination numbers were determined from cleared thin sections of the leaf lamina mounted in glycerin solution (5%). With the aid of a camera lucida and stage micrometer, epidermal cells, stomata, palisade cells, veinlet terminations and vein-islets per square millimeter were determined. For each parameter, the various cell types were counted from five different fields of view and the average was recorded. Standard procedures were followed according to established methods (Khandelwal, 2008, Komlaga et al., 2014).

**Powder Microscopy of Leaf and Stem Bark**

Small amounts of powdered samples were placed on a glass slide mounted in 5% glycerin or stained with N/50 iodine for the observation of starches or 0.1% phloroglucinol plus a drop of concentrated HCl for the observation of lignified cells. Ergastic content and plant cell types were observed at various magnifications and photomicrographs were taken (Evans, 2009).

**Physicochemical analysis**

Physicochemical parameters including ash values (total ash, water soluble ash, acid insoluble ash), pH of 1% aqueous and alcoholic extracts and extractive values (petroleum ether, ethyl acetate, ethyl alcohol, methanol and water soluble extractive values) were determined for both leaves and stem bark (Asante-Kwata et al., 2019). The experiment was carried out in triplicate and the results expressed as the mean ± standard deviation.

**Fluorescence analysis**

Extracts of different organic solvents were prepared and the characteristic fluorescence colours emitted by the solutions were observed in visible light, under short and long UV light and recorded. Also, small amounts of the powdered plant materials were placed on clean non-fluorescence glass slides and few drops of dilute acid or alkali added and subsequently observed in the same manner (Gupta et al., 2012).

**Phytochemical screening**

The dried powdered plant materials were tested for the presence major groups of secondary metabolites according to standard methods (Evans, 2009).

**Biological activity studies**

**Acute toxicity test**

Acute toxicity studies were performed following a previously described method (Abotsi et al., 2017). Briefly Sprague-Dawley rats of both sexes (n = 5/group) were orally administered with 300, 1000 and 3000 mg/kg of the leaf (APL) and stem bark (APSB) extracts after an overnight fast (water ad libitum). A control group was given normal saline (10 mL/kg). The animals were closely monitored for gross behavioural changes or death at 0, 15, 30, 60, 120 and 180 min and also 24 h and 14 days after extract administration. This was to determine the doses of extract that will be safe for administration to rats without causing any adverse effect or death.

**Anti-inflammatory activity**

The anti-inflammatory activity of the extracts was determined by the carrageenan-induced pedal oedema in the chick model (Roach and Sufka, 2003) with some modification (Mireku et al., 2014) as previously described. To induce oedema, 10 μL of carrageenan (2% solution in normal saline) was injected into the sub-plantar tissue of the right footpad of the chicks. Footpad diameter was measured before carrageenan injection. An hour post carrageenan injection, chicks (n=6) were treated with APL and APSB suspended in normal saline (30, 100 and 300 mg/kg p.o.). Dexamethasone (0.3, 1 and 3 mg/kg i.p.) and diclofenac (10, 30 and 100 mg/kg i.p) were used as standard and normal saline (10 ml/kg p.o.) as the negative control. Footpad diameter was subsequently measured at hourly interval for 5 h using Vernier callipers (model: Z22855, Millomex Ltd., Bedfordshire, UK). Raw scores from the foot pad diameters were normalized as percentage change in foot pad size from their values at time 0 calculated by:

% Change in foot pad size = \( \frac{(f_t - f_0)}{f_0} \times 100 \)

Where 0 is foot pad size at time 0 and ft is foot pad size at time t (hourly time intervals).

**Antipyretic assay**

The antipyretic activity of the extracts was determined by the baker’s yeast-induced hyperthermia method as previously described (Boakye-Gyasi et al., 2008). Prior to the experiments the rectal temperatures (T_R) of test animals were taken by inserting a lubricated digital thermometer about 3 cm into the rectum of the rat and animals with temperatures between 36 and 37°C were selected for the antipyretic activity studies. After taking the basal temperatures, animals were injected with pyrogenic dose of baker’s yeast (0.135 g/kg, i.p.). Animals that showed an increase in temperature of not less than 0.5°C were used for the experiment. Animals were randomly divided into groups with five rats in each group and were administered extracts (30, 100 and 300 mg/kg p.o.), the antipyretic paracetamol (10, 50 and 150 mg/kg) or normal saline (10 ml/kg p.o.). A naive control group which was not treated with baker’s yeast was included and given 0.5 ml normal saline (p.o). The change in T_R was monitored hourly for 8 h.

**Statistical analysis**

All statistical analysis was performed using GraphPad Prism for Windows Version 6 (GraphPad Prism Software). All data were presented as the mean ± S.E.M. The time-course curves from all experiments were subjected to two-way (treatment ×time) repeated measures analysis of variance (ANOVA). The total oedema and pyrexia scores for each treatment group were calculated in arbitrary units as the area under the curve (AUC). Differences in AUCs were analysed and multiple comparisons between treatment groups determined by the Newman-Keul’s post hoc test. P < 0.05 was considered statistically significant.

**RESULTS**

**Macroscopic features of A. pterocarpoides**

The leaf of A. pterocarpoides is petiolated, oddly pinnate
compound with averagely 11 to 15 leaflets arranged in a sub-opposite manner on a rachis. The leaflets measure approximated 5.3 to 16.2 cm in length and 3.2 to 6.4 cm in width (Figure 1a). They are oblong with entire margin, acuminate apex and a more or less obtuse base. They have sub-marginal and reticulate venation and a papery feel. The lower surface is pubescent and light green, while the upper surface is glabrous and deep green in colour. It tastes slightly bitter.

The slashed stem bark is thick and curved. Its outer surface is greenish grey, scaly and rugged with cork tissue, lichens and mosses (Figure 1b). The inner surface is reddish-brown with longitudinal striations (Figure 1c). Its fracture is fibrous internally and horny towards the outer surface. The odour is characteristic and the taste, bland.

Microscopic features of *A. pterocarpoides*

*Microscopy of the leaf lamina*

The epidermal cells on both the lower and upper surfaces of the leaf lamina are highly convoluted (Figure 2a). The leaf is hypostomatus with paracytic stomata occurring among wavy-walled epidermal cells on the abaxial surface (Figure 2a). Also present on the lower surface are unicinate trichomes, measuring about 15-23 µm,
rooted on the midrib and veins (Figure 2b).

**Microscopy of Midrib**

Microscopy of the transverse section (T/S) of the midrib displays an impressed midrib with a bulging ventral base and a slight protrusion on the dorsal surface (Figure 3a). The vascular bundle is conjoint and surrounded by a zone of lignified pericyclic sclerenchymatous sheath forming a ring around the pith (Figure 3b). Both non-glandular (covering) and glandular trichomes occur on the epidermis. The glandular trichomes have multicellular heads and unicellular stalks (Figure 3c) and the covering trichomes are uniseriate and multicellular with blunt apices and smooth walls (Figure 3d). The ventral surface of the midrib is covered with a thick cuticle followed by a single layer of epidermal cells and 3-4 layers of irregularly shaped collenchyma cells, beneath which large loosely arranged parenchyma cells are found (Figure 3e).

**Microscopy of leaf petiole**

The transverse section of the petiole is circular in shape with numerous covering and glandular trichomes on the surface (Figure 4a). The vascular bundle is kidney-shaped, conjoint and collateral (Figure 4b). It is surrounded by a lignified sclerenchymatous sheath which together forms an incomplete ring around the pith. Outside the sclerenchyma ring is the cortex made of the parenchyma and the collenchyma cells embedded with prismatic calcium oxalate crystals (Figure 4c).

**Quantitative microscopy**

Table 1 presents leaf surface constants that are peculiar to and useful for identification of *A. pterocarpoides*. Values are presented as the average ± SEM of three determinations.

**Powder microscopy**

**Leaf powder**

Microscopy of the leaf powder showed lignified sclereids, clothing trichomes, non-lignified fibres, prismatic calcium oxalate crystals and starch granules (Figure 5).

**Stem bark powder**

Microscopy of stem bark powder showed the presence of starch grains, isolated stone cells, prismatic calcium oxalate crystals, lignified fibres and clustered brachysclereids (Figure 6).

**Fluorescence analysis**

The fluorescence characteristics of various solvent
Figure 4. Transverse section (TS) of the petiole (a) displaying (b)- lignified bean-shaped vascular bundle (c)- prismatic calcium oxalate crystals and trichomes [vb- vascular bundle; cr- calcium oxalate crystal; tr-trichome].

Table 1. Leaf surface constants of *A. pterocarpoides.*

| Leaf surface determinant             | Mean ± SEM   |
|--------------------------------------|--------------|
| Stomata number                       | 14.60 ± 0.65 |
| Stomata index                        | 16.67 ± 0.93 |
| Palisade ratio                       | 4.25 ± 0.23  |
| Vein islet number                    | 25.00 ± 0.39 |
| Veinlet termination number           | 5.90 ± 0.35  |

Figure 5. Microscopy of the leaf powder showing (a)- stone cells; (b)- covering trichome; (c)- lignified fibre; (d)- prismatic calcium oxalate crystal; (e)- aggregated starch granule.
extracts of both leaf and stem bark was observed under different electromagnetic wavelengths as shown in Table 2. The extracts in various solvents, acidic and alkali conditions produced various shades of colours under visible and UV light. In acidified medium neither the leaf nor stem bark extracts fluoresced under either short (254 nm) or long (365 nm) wavelengths of UV radiation. However, in basified medium both leaf and stem bark extracts produced yellow and pale yellow fluorescence respectively at 365 nm but no fluorescence at 254 nm wavelength of UV.

Phytochemical screening

Phytochemical analysis of the leaf and stem bark of *A. pterocarpoides* revealed the presence of major classes of secondary metabolites presented on Table 3.

Physicochemical analysis

Various physicochemical parameters including the total, water soluble and acid insoluble ash contents, solvent extractives as well as the pH of edible extracts (that is, water and 70% ethanol extracts) were determined and the results presented on Table 4.

Biological activity studies

*Acute toxicity*

No signs of toxicity or death was recorded in rats during the period of experiment, after 24 h and 14 days of extract administration.

**Anti-inflammatory activity**

Injection of 2% carrageenan resulted in moderate acute inflammation observed as foot oedema in the 7-day old chicks. Figures 7 and 8 present the time course curves and total oedema responses expressed in arbitrary units as the area under the curve (AUCs) for the effect of APL, APSB, diclofenac and dexamethasone on the carrageenan-induced foot pad oedema. From the time course curve of the untreated group (negative control), oedema was highest during the 2rd to 3rd hours after which it slowly declined in the course of the experiment.

Two-way ANOVA of the time course curves (treatment × time) revealed significant reduction in the mean maximal foot pad oedema for APL ($F_{3, 14} = 5.643, P = 0.0078$), APSB ($F_{3, 16} = 7.889, P = 0.0022$), diclofenac ($F_{3, 12} = 7.018, P = 0.0056$) and dexamethasone ($F_{3, 12} = 10.11, P = 0.0013$). APL and APSB (30-300 mg/kg, p.o.) caused significant reduction in total foot oedema with a maximal effect of $76.65 \pm 6.27\%$ and $77.04 \pm 2.53\%$ respectively (Figure 7a-d). The reference drugs, dexamethasone and diclofenac also significantly reduced oedema with maximal effects of $78.81 \pm 9.29\%$ and $77.58 \pm 2.85\%$ respectively at their highest doses (Figure 8a-d). Figure 9 compares the dose-response relationship among the test extracts and reference drugs and indicates the anti-inflammatory activity of the samples to be APL$<$ APSB$<$ Diclofenac$<$ Dexamethasone in order of increasing potency. The ED$_{50}$ values obtained from the
Table 2. Fluorescence characteristics of the dry powders of the leaves and stem bark of *A. pterocarpoides* in various solvents/media.

| **A. pterocarpoides leaves** | **Visible light** | **UV-254 nm** | **UV-365 nm** |
|-----------------------------|-------------------|----------------|---------------|
| Powder + Water              | Brown             | Pale yellow    | Pink          |
| Powder + Ethanol 95%        | Green             | Reddish-brown  | Pink          |
| Powder + Methanol           | Dark green        | Pale yellow    | Pink          |
| Powder + Ethyl acetate      | Light green       | Reddish-brown  | Pink          |
| Powder + Chloroform         | Olive-green       | NF             | Pink          |
| Powder + Petroleum ether    | Yellowish-green   | Reddish brown  | Pink          |
| Powder + HCl                | Green             | NF             | NF            |
| Powder + KOH                | Greenish-brown    | NF             | Yellow        |

| **A. pterocarpoides stem bark** | **Visible light** | **UV-254 nm** | **UV-365 nm** |
|---------------------------------|-------------------|----------------|---------------|
| Powder + Water                  | Reddish brown     | Pale Yellow    | Pale yellow   |
| Powder + Ethanol 95%            | Light brown       | Reddish-brown  | Pale yellow   |
| Powder + Methanol               | Amber             | Brick red      | Purple        |
| Powder + Ethyl acetate          | Light brown       | NF             | Pale yellow   |
| Powder + Chloroform             | Reddish-brown     | Reddish-brown  | Pale yellow   |
| Powder + Petroleum ether        | Light brown       | NF             | Pale yellow   |
| Powder + HCl                    | Reddish-brown     | NF             | NF            |
| Powder + KOH                    | Yellowish-brown   | NF             | Pale yellow   |

NF-No fluorescence.

Table 3. Phytochemical screening of leaf and stem bark of *A. pterocarpoides*.

| Constituent      | Leaf | Stem bark |
|------------------|------|-----------|
| Saponins         | +    | +         |
| Tannins          | +    | +         |
| Alkaloids        | -    | +         |
| Flavonoids       | +    | -         |
| Reducing Sugars  | +    | +         |
| Triterpenoids    | +    | +         |
| Coumarins        | +    | +         |
| Steroids         | +    | +         |

(+)- Detected, (–)- Not Detected.

non-linear regression curves are presented on Table 5.

**Antipyretic activity**

The change in rectal temperature (T_R) with time resulting from injection of the pyrogenic dose (0.135 mg/kg, i.p.) of baker’s yeast for all treatment groups is presented in Figure 10. The mean rectal temperature increased from 36.51 ± 0.37°C to 38.53 ± 0.61°C for all treatment groups except the saline treated group which showed no significant change in rectal temperature. The increase in temperature was maintained 4 h post pyrexia induction. Two-way ANOVA of the time course curves (treatment × time) revealed that APL (30, 100, 300 mg/kg, p.o.) and APSB (30, 100 and 300 mg/kg, p.o.) caused a significant dose-dependent reduction in pyrexia in rats at all treatment doses [APL: F_4, 20 = 40.81, P < 0.0001; APSB: F_4, 20 = 31.31, P < 0.0001, Figure 10 a-d]. Treatment with 300 mg/kg APL and APSB induced a significant (P< 0.05) inhibition of pyrexia by 79.37 ± 5.27 % and 63.41 ± 0.66 % respectively. Similarly, the standard antipyretic drug
Table 4. Physicochemical analysis of the leaves and stem bark of *A. pterocarpoides*.

| Test                  | Results                  |
|-----------------------|--------------------------|
|                       | Leaf                     | Stem bark                |
| Ash value (g)         |                          |                          |
| Total ash             | 4.00 ± 0.62              | 2.84 ± 0.29              |
| Acid insoluble        | 0.70 ± 0.13              | 0.28 ± 0.03              |
| Water soluble         | 1.25 ± 0.02              | 2.30 ± 0.61              |
| Extractive value (%)  |                          |                          |
| Aqueous extract       | 10.67 ± 0.67             | 12.00 ± 1.00             |
| Ethanol extract       | 14.33 ± 0.33             | 6.33 ± 0.88              |
| Ethyl acetate extract | 19.67 ± 1.20             | 11.00 ± 0.58             |
| Petroleum ether extract | 26.67 ± 0.67         | 10.33 ± 1.20             |
| pH                    |                          |                          |
| Aqueous extract       | 6.31                     | 6.03                     |
| Ethanol extract       | 6.12                     | 5.97                     |

Figure 7. Effect of curative treatment of APL and APSB on the time course curve (a-b) and total oedema response (AUC) (c-d) in carrageenan-induced oedema in chicks. Data expressed as mean ± SEM (n=5) *P<0.05, **P<0.01 as compared to negative control using one-way ANOVA. [APL- MeOH extract of *A. pterocarpoides* leaves; APSB- MeOH extract of *A. pterocarpoides* stem bark].

Paracetamol (15, 50 and 150 mg/kg, p.o.) attenuated pyrexia significantly (*F*₄,₂₀ = 85.74, *P < 0.0001) in a dose-dependent manner (Figure 10e-f) with much higher inhibition of 89.22 ± 2.48% at 150 mg/kg. The ED₅₀ values obtained from non-linear regression were 70.95 ± 9.24 mg/kg (APL), 27.02 ± 1.96 mg/kg (APSB) and 32.47 ± 3.03 mg/kg (paracetamol). The mean percentage reduction in rectal temperatures for the various treatment
DISCUSSION

The leaves and stem bark of *A. pterocapoides* are used traditionally for the treatment of inflammatory conditions and malaria fever among others. This study provided standard parameters for the correct identification and quality control of the plant and demonstrated the anti-inflammatory and anti-pyretic activities of the leaves and stem bark in animal models.

Figure 8. Effect of curative treatment of diclofenac and dexamethasone on time course curve (a-b) and total oedema response (AUC) (c-d) in carrageenan-induced oedema in chicks. Data expressed as mean ± SEM (n=5) *P<0.05, **P<0.01 as compared to negative control.

Figure 9. Log dose versus response curve for APL, APSB, diclofenac and dexamethasone in the carrageenan-induced pedal oedema assay [APL- MeOH extract of *A. pterocapoides* leaves; APSB- MeOH extract of *A. pterocapoides* stem bark].

groups is summarized on Table 6.
Table 5. ED$_{50}$ values of *A. pterocarpoides* stem bark and leaf MeOH extracts in the carrageenan-induced pedal oedema assay.

| Drug                  | APL          | APSB         | Diclofenac | Dexamethasone |
|-----------------------|--------------|--------------|------------|---------------|
| ED$_{50}$ (mg/kg)     | 225.3 ± 31.25| 178.9 ± 29.57| 18.87 ± 2.23| 0.7437 ± 0.08 |

APL- MeOH extract of *A. pterocarpoides* leaves; APSB- MeOH extract of *A. pterocarpoides* stem bark.

Figure 10. Effect of APL, APSB and paracetamol treatment on time course curve and total antipyretic response (AUC) in baker’s yeast induced pyrexia in rats. Data is expressed as mean ± SEM (n = 5) *P<0.05, ***P<0.0001 as compared to negative control using One-way ANOVA. [APL- MeOH extract of *A. pterocarpoides* leaves; APSB- MeOH extract of *A. pterocarpoides* stem bark].

Standardization of medicinal plant materials is important as it ensures reproducible quality, safety and efficacy of herbal products (Asante-Kwatia et al., 2019). The macroscopic parameters provided for *A. pterocarpoides*
will enable easy identification in its natural habitat. The microscopic detail of the internal cellular structures will also ensure strict distinction between closely related species. Among the leaf surface parameters determined, the stomatal index is generally constant and not affected by age of plant, size of leaf, geographical location and environmental conditions (Baidoo et al., 2019) and can be used for distinguishing other *Amphimias* species. Crude drugs are usually processed into powders for easy packaging, transportation, and commercialization (Evans, 2009). Physicochemical parameters such as ash content and extractive values were determined for the powdered samples. A high total ash, water soluble or acid insoluble ashes is usually indicative of possible contamination with inorganic salts (including earthy material) or poor preparation (Folashade et al., 2012). Thus any significant deviation from these values may suggest contamination. From the extractive content experiment, non-polar solvents i.e. petroleum ether and ethyl acetate had high extractive power for the leaves while polar solvents including water and ethanol were the best solvents for maximum extraction of constituents from the stem bark. This may suggest that the stem bark has more polar constituents and vice versa for the leaves. The different characteristic fluorescence observed for various extracts of the leaf and stem bark of *A. pterocarpoides* is reproducible and can serve as adjunct parameter in authenticating this plant (Chase and Pratt, 1949). It is important to determine the pH of edible solutions of plant extracts to ensure that crude preparations are not irritative to the gastrointestinal tract (GIT) (Asante-Kwataia et al., 2019). Water and alcohol are mostly used for oral preparations of herbal medicines. The pH of the aqueous and alcoholic extracts of both leaves and stem bark were weakly acidic ranging between 5.9 and 6.3. This implies that traditional aqueous and alcoholic preparations of *A. pterocarpoides* are less likely to cause GIT irritation when consumed.

Acute oral toxicity studies suggested that the leaf (APL) and stem bark (APSB) extracts were safe at a limit test dose of 3000 mg/kg. No death was recorded and physical observation revealed no sign of toxicity such as abnormal behavioural patterns, trembling, diarrhoea, lack of locomotor activity, sleep or coma in rats. This result is consistent with a previous report that estimated the LD_{50} of the methanol extract of the stem bark to be more than 5000 mg/kg (Tchoumtchoua et al., 2014). The anti-inflammatory activity of the extracts was validated in the carrageenan-induced oedema assay which is a classical model of acute inflammation validated in rodents and chicks (Roach and Sufka, 2003; Whiteley and Dalrymple, 2001). The acute inflammatory cascade effectuated by carrageen has been proposed to be a biphasic event characterized by the release of histamine, serotonin and bradykinins in the early phase (0-2 h) and a latter accelerating phase of swelling which results from increased post-capillary venule permeability, fluid exudation and visible oedema due to the over production of prostaglandins and activation of cyclooxygenase-2 (COX-2) (Necas and Bartosikova, 2013). From the experiment, APL and APSB reduced the foot pad oedema from the first hour and subsequently throughout the 5 h period of observation, implying that the extracts were active in both phases of the induced inflammation. Though the exact mechanism of anti-inflammatory action of APL and APSB is not yet known, it can be contemplated that the extracts have influence on the arachidonic acid pathway interrupting the synthesis, release or action of any of the mediators involved with carrageenan induced inflammation. Among the two extracts, APSB demonstrated the highest anti-inflammatory effect and its effect was dose-dependent. Interestingly the anti-inflammatory effect of the leaf extract was not dose-dependent as the highest effect was elicited at 100 mg/kg. This is possible considering the fact that crude extracts contain several phyto-constituents some of which could exhibit pro-inflammatory effects when in high concentrations, thereby masking the anti-

### Table 6. Mean percentage reduction in rectal temperature (T_r).

| Drugs                                      | Dose (mg/kg, p.o.) | % Reduction in T_r |
|--------------------------------------------|--------------------|--------------------|
| APL                                        | 30                 | 66.90 ± 5.87       |
|                                            | 100                | 73.54 ± 2.03       |
|                                            | 300                | 79.37 ± 5.27       |
|                                            | 30                 | 56.29 ± 5.19       |
| APSB                                       | 100                | 61.47 ± 4.02       |
|                                            | 300                | 63.41 ± 0.66       |
| Paracetamol                                 | 10                 | 78.10 ± 2.32       |
|                                            | 100                | 87.50 ± 2.58       |

APL- MeOH extract of *A. pterocarpoides* leaves; APSB- MeOH extract of *A. pterocarpoides* stem bark.
inflammatory effect (Danquah et al., 2011). The anti-inflammatory effect of both extracts was lower than the standard drugs diclofenac and dexamethasone.

The antipyretic activity of APL and APSB was assessed by the baker's yeast-induced fever model which is a reliable method for inducing a clear-cut reversible fever in animal models (Tomazetti et al., 2005). The yeast-induced fever is considered a pathogenic fever where yeast (a lipopolysaccharide component of Gram negative bacteria) acts as an exogenous pyrogen, inducing the release of pro-inflammatory cytokines and eventually the release of local prostaglandins mainly PGE$_2$ via the arachidonic acid pathway. The role of PGE$_2$ in febrile responses cannot be overemphasized. Thus antipyretic activity is a characteristic of drugs or compounds with inhibitory effects on the biosynthesis of prostaglandins (Romanovsky et al., 2005; Walter et al., 2016). APL and APSB elicited remarkable hypothermal activity against yeast-induced pyrexia in rats though their effect was lower than the standard antipyretic paracetamol. Following the anti-inflammatory activity observed by APL and APSB in the carrageenan-induced oedema, the antipyretic activity exhibited by the extracts further reiterate their possible influence on the biosynthesis or action of prostaglandins.

Preliminary phytochemical screening of the leaves and stem bark of *A. pterocarpoides* revealed the presence of tannins, flavonoids, coumarins, triterpenes, phytosterols and saponins. Previous studies also reported the presence of isoflavonoids with antioxidant and antimicrobial activity in the roots of *A. pterocarpoides* (Saah et al., 2013). Most of these classes of plant secondary metabolites are known to attenuate the damaging effect of inflammation and have been shown to contribute to the anti-inflammatory and anti-pyretic activity of many medicinal plants (Mireku et al., 2015; Armah et al., 2015; Shah and Seth, 2010; Asante-Kwatia et al., 2020). Triterpenoids inhibit the infiltration of inflammatory mediators such as prostaglandins hence prevents fluid exudation into affected tissues (Rios et al., 2000; Zhu et al., 2018). Serafini et al. (2010) demonstrated that flavonoids exhibit anti-inflammatory activity by blocking the synthesis of various pro-inflammatory mediators including eicosanoids, cytokines, adhesion molecules and C-reactive proteins (Serafini et al., 2010). Isoflavonoes were also shown to exhibit anti-inflammatory effect by the down regulation of cytokine mediated signal transductions and by reducing the production of interleukins IL- β, IL-6, nitric oxide (NO), and prostaglandin E2 (PGE$_2$)(Yu et al., 2016; Park et al., 2007). The observed anti-inflammatory and antipyretic effect of the plant may thus be attributable to the presence of these phyto-constituents.

**Conclusion**

The diagnostic features obtained for the leaves and stem bark of *A. pterocarpoides* are useful for the authentication and quality control of the plant. The anti-inflammatory and antipyretic activities of the leaves and stem bark of *A. pterocarpoides* have been demonstrated in this work supporting the folkloric use of the plant in the treatment of inflammatory conditions and fever.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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