Anti-inflammatory and Antioxidant Activities of Hunteria umbellata Seed Fractions

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Abstract: Background: Decoction of Hunteria umbellata seeds is highly valued in African herbal medicine in the management of inflammation, diabetes and obesity. This study evaluated the anti-inflammatory and antioxidant activities of crude (HU) butanol (HU_B) and alkaloid (HU_A) fractions of the water seed extract of Hunteria umbellata, in addition, to determining the phenolic content of HU and Hu. Materials and methods: The acute anti-inflammatory activity of 50 mg kg⁻¹ of each of the seed fractions was evaluated in carrageenan- and formalin-induced oedematous Wistar rats. The fraction’s antioxidant activities were evaluated using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical, superoxide anions and nitric oxide scavenging methods, in addition, to determining the phenolic contents of the fractions using standard procedures. Results: Results showed that HU, HU electorate (HU_elect) and HU_A significantly (p<0.05 and p<0.001) inhibited carrageenan- and formalin-induced inflammation in the rats. Similarly, HU, HU_elect at 0.2-0.8 mg mL⁻¹ exhibited significant (p<0.05 and p<0.001) DPPH free radical, superoxide anion and nitric oxide scavenging activities with the most significant effect recorded for HU_elect. Their proanthocyanidin contents were estimated to be 38.90±1.67 and 53.67±1.12 mg g⁻¹ of dry extract, respectively while their total flavonoid contents were estimated to be 0.50±0.03 and 11.78±1.47 mg g⁻¹ of dry extract, respectively. Also, their total phenolic contents were estimated to be 39.68±2.56 and 97.12±3.32 mg g⁻¹ of dry extract. Conclusion: The anti-inflammatory activity of HU and its butanol fraction is attributed to its alkaloid content which was partly mediated via its anti-oxidant mechanism.

Key words: Hunteria umbellata (K. Schum.) Hallier f., aqueous seed fractions, anti-inflammatory activity, free radical scavenging/antioxidant mechanism

INTRODUCTION

Hunteria umbellata (K. Schum.) Hallier f. (family: Apocynaceae) is a West African glabrous tree which is known as Demouain (in French) (Boone, 2006) and Abeere (in Yoruba, Southwest Nigeria) (Ibeh et al., 2007; Adeneeye and Adeyemi, 2009a). In African folk medicine, Hunteria umbellata plant has wide therapeutic applications including the local treatment of pain, swellings, infections, gastric ulcers, liver diseases, diabetes mellitus, obesity and management of labour at term (Ejimadu and Falodun, 2002; Falodun et al., 2006; Adeneeye and Adeyemi, 2009a). In Germany, bitter tonics made from Hunteria umbellata are used for reducing high blood pressure and blood lipids (Boone, 2006). Recently, the antihyperglycaemic effects of the aqueous seed extract in different experimental models of diabetes (Adeneeye and Adeyemi, 2009a; b; Igbe et al., 2009a) and its anti-obesity and anti-hyperlipidaemic effects (Adeneeye et al., 2010) were reported. Acute and chronic oral toxicity studies have equally shown the aqueous seed extract of the plant to be relatively safe in rats pre-treated with the extract (Adeneeye et al., 2010b).

In African traditional medicine, water decoction of the pulp and dried seeds of Hunteria umbellata is reputed for the management of arthritic swellings (Igbe et al., 2009b). Recently, the antipyretic and analgesic effects of the fruit pulp extract of the plant were validated (Igbe et al., 2009b). Despite the historical and extensive folkloric use of the water infusion of the plant seed in the management of arthritic swelling, there is no evidence to back up this folkloric claim. This forms the basis of the current study.

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which evaluates the anti-inflammatory effect and mechanism(s) of HU and its fractions in wistar rats. Also, bearing in mind the strong association between inflammation and ROS generation (Schreckinger et al., 2010), the current study evaluates the in vitro antioxidant activity of the aqueous seed extract of Hunteria umbellata (HU), its butan-1-ol fraction (HUₘ), and its total alkaloid fraction (HUₖ) using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical, superoxide anion and nitric oxide scavenging methods. In addition, the polyphenol derivative contents of the extracts were also estimated using standard procedures.

**MATERIALS AND METHODS**

**Collection of plant materials:** Dried seeds of Hunteria umbellata were purchased from a retailer of herbal produce in Jakande Estate, Oke-Afa, Isolo, Lagos State, Nigeria, in December 2009. These were identified and authenticated by Mr. T.K. Odeowo (Chief Superintendent of Taxonomy) at the Herbarium of the Department of Botany and Microbiology, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria. The seeds were de-coated of their light-brown coatings, gently rinsed in tap water and completely air-dried under room temperature (30±2°C) for 4 weeks protected from direct heat or sunlight. The seeds were kept in air-and water-tight container and stored in the refrigerator.

**Aqueous extraction of hunteria umbellata seeds:** One hundred gram of the dried seeds was pulverized to white-to-light brown fine powder using a domestic blender. Thirty gram of the fine powdered sample was macerated in 500 mL of distilled water in a 1 L Pyrex beaker and kept in the refrigerator at 4°C for 72 h. After 72 h, the homogenate was then continuously stirred for 6 h before it was rapidly filtered through a piece of clean white cloth. The filtrate was then completely dried to an aromatic, deep brown solid residue in an aerated oven preset at 40°C. The weight of the solid residue left behind was 23 g, giving a yield of 23.0% (w/w). The residue was stored in air-and moisture-tight container which was kept in a refrigerator. From this, a fresh stock was reconstituted in distilled water at a concentration of 100 mg mL⁻¹, whenever needed.

**Solvent partitioning of HU:** Thirty gram of the extract was suspended in 100 mL of distilled water. The solution was then transferred into 5 L burette before it was partitioned using between 1 to 1.5 L of different partitioning solvents (diethyl ether, chloroform, acetyl acetate and butan-1-ol) in the order of their increasing solubility gradients. The fraction obtained with each partitioning solvent was concentrated in vacuo using rotary evaporator (Büchi Rotavapor® Model R-215, Switzerland) with Vacuum Module V-801 EasyVac®, Switzerland set at a revolution of 70 rpm and a temperature of 35°C. The solid residue and the concentrate of each fraction were then transferred to an aerated oven preset at 35°C for complete dryness. The residues left after oven drying were then weighed. This procedure was repeated thrice and each residue was pooled together and stored in clean and dry, water and air-proof containers and preserved in the refrigerator until required for experimentation.

**Determination of DPPH scavenging activity of HU and its fractions:** The effect of HU, its butanol and alkaloid fractions was estimated using the method of Liyana-Pathirana and Shadidi (2005). A solution of 0.135 mM 1,1-Diphenyl-2-picrylhydrazyl (DPPH) (Sigma Aldrich, St. Louis, USA) in methanol was prepared and 1.0 mL of this solution was mixed with 1.0 mL of methanol containing 0.2-1.0 mg of each extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature or 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid (Sigma Chemicals Co., St. Louis, USA) equally prepared at same concentration was used as the reference drug. The experiment was conducted in triplicate. The ability to scavenge DPPH radical was calculated by the following equation:

\[
\text{DPPH radical scavenging activity (\%)} = \frac{(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{std}})}{(\text{Abs}_{\text{sample}})} \times 100
\]

Where:

- \(\text{Abs}_{\text{sample}}\) = Absorbance of DPPH radical + methanol
- \(\text{Abs}_{\text{std}}\) = Absorbance of DPPH radical + sample extract/standard

**Determination of superoxide anion and nitric oxide scavenging activities of HU and its fractions:** Superoxide anion and nitric oxide scavenging activities of HU and its fractions were evaluated using the methods of McCord and Fridovich (1969) and Sreejayan and Rao (1997), respectively. In both assaying methods, quercetin was used as the standard drug.

**Quantitative determination of polyphenolic contents of HU and \(\text{HU}_{\text{m}}\):** The total flavonoids, procyanidocyanidins and phenolic contents in HU and \(\text{HU}_{\text{m}}\) were determined using standard procedures described below:

**Determination of total flavonoid content in HU and \(\text{HU}_{\text{m}}\):** Total flavonoids in HU and \(\text{HU}_{\text{m}}\) were estimated using the method of Ordonez et al. (2006) with a modification and as
adopted by Aidedapo et al. (2008). To 1 mL of crude HU and HU, equivalent 1 mL of 2% aluminium chloride in ethanol solution was added. After 1 h of incubation at room temperature (28°C) for colour development, the absorbance was measured at 420 nm using Unico 2100 Spectrophotometer (United Products and Instruments Inc., Shanghai, China). A golden yellow colour indicated the presence of flavonoids. Total flavonoids contents were calculated as rutin hydrate (minimum 98%) (Sigma Chemicals Co., St. Louis, MO, USA) equivalent from the calibration curve $y = 44.77x + 0.1036$, $R^2 = 0.9812$, where, $x$ was the absorbance and $y$ was the rutin equivalent (mg g$^{-1}$).

**Determination of total proanthocyanidin contents in HU and HU**: Total proanthocyanidins (tannin) contents in HU and HU, were estimated by method of Sun et al. (1998) and as adopted by Sofidiya et al. (2008). The 0.5 mL of 50 mg L$^{-1}$ of the extract was mixed in 3 mL of 4% vanillin-methanol solution and 1.5 mL concentrated hydrochloric acid and the mixture was allowed to stand for 15 min at room temperature (28°C) for colour development. The absorbance was measured at 500 nm using Unico 2100 Spectrophotometer (United Products and Instruments Inc., Shanghai, China). Total proanthocyanidins contents were calculated as catechin hydrate (minimum 98%) (Sigma Chemicals Co., St. Louis, MO, USA) equivalent (mg g$^{-1}$) using the following equation based on the calibration curve: $y = 3.95x - 0.0077$, $R^2 = 0.9218$ where, $x$ was the absorbance and $y$ was the catechin equivalent (mg g$^{-1}$).

**Determination of total phenols in HU and HU**: Total phenol contents in HU and HU, were determined by the modified Folin-Ciocalteau method of Wolfe et al. (2003) and as adopted by Sofidiya et al. (2008). An aliquot of each of HU and HU, was mixed with 2.5 mL Folin-Ciocalteau reagent (previously diluted with distilled water, 1:10 v/v) and 2 mL of (75 g L$^{-1}$) of sodium carbonate. The tubes were vortexed for 15 seconds and allowed to stand for 30 min at 40°C for colour development. Absorbance was measured 765 nm using Unico 2100 Spectrophotometer (United Products and Instruments Inc., Shanghai, China). Total phenolic content was expressed as mg g$^{-1}$ rutin equivalent using the following equation based on the calibration curve: $y = 8.6x + 0.2004$, $R^2 = 0.9814$, where $x$ was the absorbance and $y$ was the rutin equivalent (mg g$^{-1}$). The amount of total phenol was calculated as rutin equivalent from the calibration curve. The experiment was replicated thrice.

**Extraction of alkaloid fraction (HU) from HU**: Ten gram of HU was suspended in 50 mL of 5% aqueous HCl acidified water and extracted four times with chloroform.

The combined acid solution was carefully basified with sodium trioxocarbonate (IV) (Na$_2$CO$_3$) solution to pH 12 and then extracted three times with chloroform (150 mL) until the solution was almost colourless and the basic solution gave negative tests with alkaloid detecting reagents (Adogoke and Alo, 1986). The chloroform solution concentrated at the room temperature (32°C) to give a sweet-smelling, dark brown solid residue weighing 0.730 g (yield: 7.3%). This procedure was repeated three more times and the residues were pooled into a tight-capped container which was stored in the refrigerator until required for experiment.

**Experimental animals**: Fifty, young adult male Wistar rats (130-150 g) were obtained from the rat colony of the Animal House, College of Medicine, University of Lagos, IIdi-Araba, Lagos, Nigeria, in the month of May, 2010, after ethical approval was obtained. Animals were housed in propylene cages, maintained under standard laboratory conditions and quarantined for 7 days to allow for acclimatization. Experimental animals were also handled and processed according to institutional international guidelines on the use of animals for experiment (United States National Institutes for Health, 1985).

**In vivo anti-inflammatory evaluation of HU and its fractions**: Carrageenan-induced paw oedema: Twenty-five young adult male Wistar rats (130-150 g) were divided into five groups of five rats per group. In all the treatment groups, rats were orally pre-treated with the extract fractions 1 h before inducing acute inflammation with single sub-plantar injection of 0.1 mL of freshly prepared 0.5% (w/v) carrageenan suspension in normal saline using a modified method of Agbaje et al. (2008). The carrageenan solution in normal saline which was injected into the sub-plantar tissue of the left hind paw of the rat served as the tested while that of the right hind paw served as the control.

Treatment of rats is as described below:

**Group I**: 10 mL kg$^{-1}$ of distilled water + 0.1 mL of 0.5% carrageenan suspension
**Group II**: 100 mg kg$^{-1}$ of aspirin + 0.1 mL of 0.5% carrageenan suspension
**Group III**: 30 mg kg$^{-1}$ of HU + 0.1 mL of 0.5% carrageenan suspension
**Group IV**: 50 mg kg$^{-1}$ of HU + 0.1 mL of 0.5% carrageenan suspension
**Group V**: 50 mg kg$^{-1}$ of HU + 0.1 mL of 0.5% carrageenan suspension
Aspirin was dissolved in distilled water and the extract fractions were dissolved in 10% Tween 20 in distilled water because of their incomplete solubility in distilled water. The paw thickness of animals in all groups was measured using vernier calipers before and after 4 h after carrageenan injection. The volume of oedema for each rat was expressed as the difference in the diameter of the rat paw before and after injection of the carrageenan.

**Formalin-induced paw oedema:** In this model of experimental inflammation, the experimental procedure was the same as described above except that single dose of 0.2 mL of 2% formalin (v/v) in distilled water was injected into the rat paw to induce the inflammation.

In both models, the increases in paw thickness and percent inhibition were calculated using the formula below as adopted by Joseph et al. (2009):

\[
\frac{P_t - P_i}{P_i} \times 100
\]

where, \( P_i \) is paw thickness at time t, \( P_t \) is initial paw thickness, \( P_i \) is the increase in paw thickness of the control group and \( P_t \) is the increase in paw thickness of the treatment groups.

**Statistical analysis:** Data were analysed using two-ways analysis of variance and the significance of the difference between the means were considered at \( p<0.05 \) and determined by student’s t-test.

**RESULTS**

**Solvent partitioning of HU:** Partitioning of HU with diethyl ether, chloroform, acetyl acetate and butan-1-ol and the eventual oven-drying of the fractions yielded 0 g of diethyl ether fraction (%yield = 0%), 1 g of chloroform fraction (%yield = 3.3%), 2 g of acetyl acetate fraction (6.6%), 4 g of butan-1-ol fraction (%yield = 13.3%) and 22 g of the solid residue (%yield = 73.3%), all of which were insoluble in water.

**Quantification of the proanthocyanidins and phenolic contents of HU and HU,:** Based on the various methods adopted in the determination of the phenolic contents in HU and HU, the linear regression analysis of calibration plots gave a correlation coefficient (R²) of 0.9218 for catechin, 0.9948 for rutin, 0.9814 for rutin used as standards in the determination of proanthocyanidins, flavonoids and total phenol, respectively. The proanthocyanidin contents of HU and HU, extract were estimated to be 38.90±1.67 and 53.67±1.12 mg g⁻¹ of dry extract, respectively while the total flavonoidal contents of HU and HU, were estimated to be 0.50±0.03 and 11.78±1.47 mg g⁻¹ of dry extract, respectively (Table 1). Also, the total phenolic contents in HU and HU, were estimated to be 39.68±2.56 and 97.12±3.32 mg g⁻¹ of dry extract (Table 1).

**DPPH free radical scavenging activities of HU and its fractions:** Using DPPH, the free radical scavenging activities of HU, HU, and HU, and ascorbic acid (the reference drug) were observed to be dose dependent with HU, having the most significant \( (p<0.05) \) free radical scavenging activity (Fig. 1). Although, initially at 0.2-0.8 mg mL⁻¹, the free radical scavenging activity of

![Fig. 1: DPPH free radicals scavenging activities of the crude aqueous seed extract (HU), butan-1-ol fraction (HU,) and alkaloid fraction (HU,) of *Hunteria umbellata* (K. Schum.) Hallier f. (Apocynaceae). Represents a significant increase at \( p<0.05 \) when compared to HU, HU, and Asc values](image)

**Table 1: Quantification of polyphenol derivatives in HU and HU,**

<table>
<thead>
<tr>
<th>Polyphenol derivatives</th>
<th>Quantity (equivalent mg g⁻¹ of dry extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HU</td>
</tr>
<tr>
<td>Total proanthocyanidins</td>
<td>38.90±1.67</td>
</tr>
<tr>
<td>(tannins)²</td>
<td></td>
</tr>
<tr>
<td>Total flavonoids²</td>
<td>0.50±0.03</td>
</tr>
<tr>
<td>Total phenols²</td>
<td>39.68±2.56</td>
</tr>
</tbody>
</table>

²Expressed as mg extract/g of dry extract, ²Expressed as mg extract/g of dry extract. ²Expressed as mg extract/g of dry extract. HU: Crude aqueous seed extract of *Hunteria umbellata*, HU,: Butan-1-ol fraction of the crude aqueous seed extract of *Hunteria umbellata*.

Fig. 2: Inhibitory effect of HU, HU₁ and HU₂f pre-treatment on in Carrageenan-Induced Oedema (CIO) and Formalin-Induced Oedema (FIO) in Wistar rat paws.

Table 2: Superoxide anion and nitric oxide scavenging activities of HU, HU₁ and HU₂f.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Superoxide anion scavenging (IC₅₀ in µg mL⁻¹)</th>
<th>Nitric oxide scavenging (IC₅₀ in µg mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HU</td>
<td>170.00±10.80</td>
<td>96.00±4.75</td>
</tr>
<tr>
<td>HU₁</td>
<td>112.00±2.50†</td>
<td>46.70±2.00†</td>
</tr>
<tr>
<td>HU₂f</td>
<td>85.00±0.45‡</td>
<td>24.00±1.40‡</td>
</tr>
<tr>
<td>Quercetin (standard)</td>
<td>85.00±0.50§</td>
<td>67.50±2.45§</td>
</tr>
</tbody>
</table>

*Represent significant decreases at p<0.05, p<0.01 and p<0.001, respectively, when compared to HU values. HU: Crude aqueous seed extract of *Houtiera umbellata*, HU₁: Butan-1-ol fraction of the crude aqueous seed extract of *Houtiera umbellata*, HU₂f: Alkaloid fraction of the crude aqueous seed extract of *Houtiera umbellata*.

HU₂f was significantly (p<0.05) higher than either HU or ascorbic acid but higher concentration, the free radical scavenging activities of extracts and reference drug became comparable (Fig. 1).

**Superoxide anion and nitric oxide scavenging activities of HU and its fractions:** HU₁ and HU₂f fractions significantly (p<0.05 and p<0.001) scavenged the superoxide generated by photo-reduction of riboflavin (Table 2). However, scavenging activity of HU₂f (IC₅₀: 85.00±0.45 µg mL⁻¹) was found to be greater than that of HU₁ (IC₅₀: 112.00±2.50 µg mL⁻¹) but relatively comparable to relative to that of the standard drug, quercetin (IC₅₀: 83.00±0.50 µg mL⁻¹) (Table 2). Similarly, HU₁ and HU₂f fractions significantly (p<0.05, p<0.001) inhibited the generation of nitric oxide from nitroprusside solution with the IC₅₀ values of 46.70±2.00 and 24.00±1.40 µg mL⁻¹, respectively when compared to HU values (Table 2).

**Anti-inflammatory activity of HU and its fractions:** Oral pre-treatment with HU, HU₁ and HU₂f resulted in significant (p<0.05, p<0.001) inhibition of inflammation caused by carrageenan and formalin injections in the treated rats. However, the % inhibition caused by 50 mg kg⁻¹ of HU, HU₁ and HU₂f was more effective than that induced by the standard anti-inflammatory agent, aspirin (Fig. 2).

**DISCUSSION**

In the current study, the anti-inflammatory and antioxidant activities of HU and its fractions were evaluated using different standard methods. Carrageenan-induced rat paw oedema has been frequently used to screen natural products with anti-inflammatory potentials (Tapas et al., 2008). Inflammation induction with carrageenan involves the activation of platelet activation factor and release of pro-inflammatory mediators such as prostaglandins, kinins, tumor necrosis factor and nitric oxide (Tan-No et al., 2006). Carrageenan induces inflammation in three distinct major phases namely: the first phase which involves the release of histamine and serotonin, release of kinins in the second phase and the activation of cyclooxygenase-2 enzyme and ultimate release of prostaglandins in the third phase (Agbaje et al., 2008). Formalin has been reported to induce inflammation via similar mechanism as described for carrageenan (Joseph et al., 2009). However, non-steroidal anti-inflammatory drugs such as aspirin, indomethacin and diclofenac are known to mediate their anti-inflammatory action via inhibition of these phases of inflammatory response (Vane and Booting, 1987; Noguchi et al., 2005). It is, thus, possible that HU and its fractions mediate their anti-inflammatory action via inhibition of these inflammation phases as well. More importantly, results indicated that the anti-inflammatory activity of HU can be attributed to its alkaloid content whose anti-inflammatory effect was profoundly higher than that of the standard anti-inflammatory drug, aspirin. Another notable result of this study is the effective free radical scavenging and antioxidant effects of HU and its fractions, with HU₂f demonstrating the most significant free radical scavenging and antioxidant activities. Literature has shown that standard antioxidant drugs such as ascorbic acid and quercetin mediate their free...
radical scavenging activities through proton donating mechanism (Wolfe et al., 2003; Adedapo et al., 2008). Thus, the results of this study suggest that the free radical scavenging and antioxidant activities of HU and its fractions may be mediated through this mechanism. Bearing in mind the strong association between inflammation and generation of ROS (Schreckinger et al., 2010), it is plausible that the anti-inflammatory activity of HU<sub>al</sub> is partly mediated via free radical scavenging and antioxidant mechanisms. Again, HU<sub>al</sub> may also account for the antioxidant activity of HU. Alkaloids and polyphenols have been reported to exhibit significant inhibitory activities on nitric oxide implicated in physiological and pathological processes as chronic inflammation (Joseph et al., 2009). The presence of alkaloids in abundance (Adegoke and Aro, 1986) may account for the antioxidant activity of HU.

In conclusion, results of this study clearly indicate that the anti-inflammatory activity of HU could be attributed to its alkaloid content which was mediated via free radical scavenging and antioxidant mechanisms.

REFERENCES


United States National Institutes for Health, 1985. Guide for the Care and Use of Laboratory Animals. DHHS Publisher, Bethesda, MD, USA.
