Analgesic and Anti-inflammatory Activities of the Stem Bark of Yellow Flamboyant
(Peltophorum pterocarpum)

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ABSTRACT: Peltophorum pterocarpum (family: Fabaceae) is an evergreen perennial crop grown in tropical
gardens. Different parts of this tree are being used for the management of many diseases. This study investigated the
acute toxicity, analgesic and anti-inflammatory potentials of n-hexane extract of P. pterocarpum stem bark. Acute oral
toxicity of n-hexane extract of P. pterocarpum stem bark was investigated using standard method. Analgesic activity
was investigated by using acetic acid-induced writhing model, using indomethacin as a reference drug. Anti-
inflammatory activity of n-hexane extract of P. pterocarpum stem bark was investigated by using formalin-induced paw
licking model, using aspirin as standard drug. In the acute toxicity study, mortality was observed at 500 and 2500 mg/kg
body weight. In the acetic acid induced writhing test, the n-hexane extract of P. pterocarpum (100 and 200 mg/kg body
weight) showed a significant reduction in the number of writhing with 55.5 % and 60 % of inhibition respectively. In
formalin-induced rat paw oedema test for acute inflammation, the n-hexane extract of P. pterocarpum in 50, 100 and
200 mg/kg body weight showed 26.00 %, 27.89 % and 32.27 % inhibition of oedema respectively after 4hours, which is
comparable to that of standard drug-aspirin (33.59 %). These results validated that the extract of P. pterocarpum
possesses significant analgesic and anti-inflammatory properties.

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Keywords: Peltophorum pterocarpum, acute toxicity, analgesic activity, anti-inflammatory activity

Free radicals are chemical species with one or more unpaired electrons such as superoxide anion (O₂⁻) and
hydroxyl radical (OH) (Dennis, 2010). Free radicals are generated as byproducts of normal aerobic
metabolism and also from reactions with xenobiotics (Singh-Deveraj and Jialal, 2005). They may either
donate an electron to or accept an electron from other molecules and hence can behave as oxidizing agents
or reducing agents (Cheeseman, 1993). Organic free radicals cause destructiveness by peroxidation of
cellular components while inorganic free radical is an important class of free radicals such as hydrogen
peroxide and superoxide anion (O₂⁻) (Gupta, 2016). Oxidative damage to DNA, proteins and other
macromolecules has been implicated in the pathogenesis of a wide variety of diseases including
diabetes, cardio vascular disease, cancer, Alzheimer’s disease, inflammatory diseases, ageing and so on
(Halliwell, 1994). Secondary metabolites are the classes of compounds which are known to display
therapeutic activities against several sicknesses in man, therefore explain the use of traditional

Proximate analysis helps to set up a definite standard for dried crude drugs in order to avoid batch-to-batch
dissimilarity and also to judge their quality (Backer and Hyne, 2012). Ash is categorized as physiological
ash which results from the plant tissue itself and non-physiological ash which is the remains after ignition
of extraneous matter (example sand and soil) (Khandelwa, 1998). The ash comprises carbonates, phosphates, silicates and silica. Acid-insoluble ash measures the occurrence of silica mainly
sand and siliceous earth. Water soluble ash is the
difference in weight between the total ash and the residue obtained after boiling the total ash in water (Backer and Hyne, 2012). Extractive values determine the quantity of active constituent extracted with dissimilar solvents in a given quantity of plant drug and use of a single solvent is the means of providing information on the value of a particular drug sample (Backer and Hyne, 2012).

Toxicology is the study of the antithetical effects of chemical, biological or physical agents on existing organisms (Radenkova-Saeva, 2008). Therapeutic plants behave as authentic because of bioactive chemical composites and the point that they are of natural source, however, does not show that they are harmless. Acute toxicity refers to the adverse effects of a substance or from frequent contacts in a short period of time (usually 24 hours) while chronic toxicity denotes the adverse health effects from repeated exposures, often at lower levels to a substance over a longer period (months or years) (International, 2008). It is normally considered dishonourable to use humans as a test subject for acute (or chronic) toxicity research (Walum, 1998).

Pain according to International Association for the Study of Pain (IASP) is unpleasant, sensory and sensitive involvement associated with actual or potential tissue destruction or described in terms of such destruction (Hassan et al., 2015). Pain is categorized into acute pain which is the body’s warning of the current damage to tissue or disease and chronic pain which is pain that lasts much longer than pain normally would, with a particular injury (Manish et al., 2010). Analgesics are the painkiller substances which act by the absence of pain without losing awareness (Manish et al., 2010). Sources of analgesic drugs are divided into synthetic drugs such as paracetamol, ibuprofen and diclofenac and natural drugs obtained from plants, animals or microbes (Agli et al., 2013).

Inflammation is a portion of the complex biological reaction of body tissues to dangerous stimuli such as pathogens, impaired cells, or irritants and is a defensive response involving immune cells, blood vessels, and molecular mediators. Five conventional signs of inflammation are heat, pain, redness, swelling and loss of function (Ferrero et al., 2007). Acute inflammation is the initial reaction of the body to harmful stimuli and is achieved by increased movement of plasma and leukocytes (especially granulocytes) from the blood into the hurt tissues while chronic inflammation is a prolonged inflammation that leads to progressive shift in the type of cell present at the site of inflammation such as mononuclear cells and is characterized by concurrent destruction and curing of the tissue from the inflammatory process (Ferrero et al., 2007). Foods that fight inflammation include tomatoes, olive oil, green leafy vegetables, nuts, fatty fish and fruits (Harvard, 2012). Nonsteroidal anti-inflammatory drugs are generally used to treat inflammation but these drugs are associated with harmful side effects such as gastric erosion, irritation, ulceration, bleeding, etc (Agli et al., 2013).

Peltophorum pterocarpum belongs to the family of Fabaceae. It is natural to tropical Southeastern Asia and a prevalently ornamental tree grown around the world (Shyamal et al., 2014). Different parts of this tree are used to treat numerous illnesses such as stomatitis, skin troubles, constipation, ringworm and its flower extract is known to be a good sleep inducer and used as cure for insomnia (Burkill, 1995). Previous investigations revealed the presence of secondary metabolites such as alkaloids, flavonoids, saponins, sterols, terpenoids, tannin and coumarins in petroleum ether and toluene extracts of the P. pterocarpum. Methanol and ethyl acetate extracts of the flowers have been reported for their analgesic activity. However, to the best of our knowledge, this is the first report of the analgesic and anti-inflammatory activities of n-hexane extract of P. pterocarpum stem bark. Consequently, the present study was designed to scientifically investigate the acute toxicity, analgesic and anti-inflammatory potentials of n-hexane extract of P. pterocarpum stem bark.

MATERIALS AND METHODS

Reagents and Chemicals: Acetic acid, hydrochloric acid, chloroform, n-hexane, aspirin, indomethacin and other chemicals and reagents used were of analytical grade.

Plant materials and Extraction: The stem bark of P. pterocarpum was collected from Ugbowo Campus of University of Benin, Benin City, Edo State, Nigeria. The plant material was identified and authenticated by Dr Akinnibosun of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, where a voucher specimen number; UBHH432 was deposited. The stem bark was rinsed with water and air-dried. The stem bark was ground to powder by means of mechanical grinder. The powdered stem bark (500 g) was extracted with 1.3 L of n-hexane by cold maceration, at room temperature for 72 hours. The extract was concentrated in-vacuo to obtain n-hexane extract with 0.38 % w/w yield.
Animals: Healthy Swiss mice (26.87±4.52 g) and healthy Wistar rats (136.50±16.19 g) of both sexes bred under standard conditions at the animal house; Department of Animal and Environmental Biology, University of Benin, Benin City, Nigeria. They were fed on a standard pellet diet (Bendel Feeds, Nigeria) and water was given ad libitum. All animal experiment conformed to the Guide for the Care and Use of Laboratory Animals published by National Academic Press (Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011).

Determination of moisture content (Anonymous, 1986): Crucibles were washed and dried in an oven at 105°C for twenty minutes. They were thereafter cooled in desiccators for ten minutes. The crude drug (10 g) was weighed and was dried at 105°C in the oven for 5 hours. The dish was transferred to the desiccators to cool, and weighed. The drying and weighing at one-hour interval was continued until a constant weight was reached.

\[ \text{% MC} = \frac{\text{weight of moisture}}{\text{weight of sample}} \times 100 \]  
Where MC = moisture content

Total ash: The powdered plant material (5 g) was weighed into previously ignited and dried crucibles. The material was spread in an even layer in the crucibles and ignited in a muffle furnace by gradually increasing the temperature to 600°C until it became ash (6 hours). The crucibles were cooled in desiccators and weighed. The total ash was calculated as the percentage of ash with reference to the powdered plant material (Backer and Heyne, 2012).

\[ \text{% total ash} = \frac{W_2 - X}{N} \times 100 \]  
Where \( W_1 \) = weight of crucible + ash; \( X \) = weight of empty crucible; \( N \) = weight of initial sample used

Acid-insoluble ash: Dilute hydrochloric acid (25 mL) was added to the crucible containing the total ash and covered with a watch glass. It was boiled gently for 5 minutes. The watch glass was rinsed with 5mL of hot water and the liquid was added to the crucible. The insoluble matter was collected on an ashless filter paper and washed with hot water. The filter paper containing the insoluble matter was transferred to the crucible, dried on a hot plate and ignited to a constant weight. The content of acid-insoluble ash was calculated as a percentage of ash with reference to the powdered plant material.

\[ \text{% Acid – insoluble ash} = \frac{W_2}{N} \times 100 \]  

Water-soluble ash: The total ash obtained was boiled for 5 minutes with 25 mL of water. The insoluble matter was collected on an ashless filter paper. It was washed with hot water and ignited for 15 minutes at a temperature not exceeding 450 °C. The total weight of insoluble matter was subtracted from the weight of the ash. This difference in weights represented the water-soluble ash. The percentage of water-soluble ash was calculated with reference to the powdered sample.

\[ \text{% Water Soluble Ash} = \frac{W_2}{W_1} \times 100 \]  

Alcohol-soluble extractive value (Khandelwal, 1998; Anonymous, 1986) (ASEV): The air-dried plant material (5 g) was coarsely powdered and macerated with 100 mL of methanol for 24 hours, with frequent stirring for the first six hours and allowed to stand for the next 18 hours. The extract was filtered rapidly taking precautions against loss of solvent. 25 mL of the filtrate was evaporated to dryness in a tarred flat bottom shallow dish and dried at 105 °C to constant weight. The weight was recorded. The percentage of alcohol-soluble extractive value was calculated with reference to the powdered sample.

\[ \text{% ASEV} = \frac{W_3 - X}{N} \times 100 \]  
Where \( W_3 \) = weight of crucible + filtrate

Water-soluble extractive value (WSEV): The powdered plant material was macerated with 100 mL of distilled water in a closed flask for 24 hours, shaking frequently for the first six hours and allowed to stand for the next 18 hours. The extract was filtered and then, 25 mL of this filtrate was evaporated to dryness in a tarred flat bottom shallow dish and dried at 105°C to constant weight and weighed. The percentage of water-soluble extractive was calculated with reference to the powdered sample.

\[ \text{% WSEV} = \frac{W_4 - X}{N} \times 100 \]  
Where \( W_4 \) = weight of crucible + Evaporated Filtrate

Acute toxicity: The acute toxicity test of the n-hexane extract of P. pterocarpon stem bark was carried out by the method described by (Lorke, 1983) with many modifications. The n-hexane extract (5, 50, 500 and 2500 mg/kg body weight) of the plant was solubilized in 0.1 mL Tween 80. The extract was agitated vigorously prior to withdrawal of injection doses to ensure even distribution of the extract. The extracts were administered intraperitoneally to the test group whereas control received only the vehicle (1 % Tween 80). Treated mice were observed for biological
reactivity continuously for the first 4 hours and a
number of survivors were noted after 24 hours of
dosing. Animals were further observed for 14 days for
any prolonged toxicity. The LD₅₀ was calculated by
using the formula:

\[ \text{LD}_{50} = (\text{HNLD} \times \text{LLD})^{1/2} \]  (7)

Where HNLD = highest non-lethal dose; LLD =
lowest lethal dose

Acetic acid-induced Writhing test: The peripheral
analgeseic activity of hexane extract of stem bark of \( P. \)
pterocarpum was determined by the acetic acid-
duced writhing inhibition method (Whittle, 1964)
with little modification. The prescreened Swiss mice
employed for the experiment were divided into five
groups. The extracts were administered orally at 50,
100 and 200 mg/kg body weight. Inhibition of
writhing in mice by the plant extract was compared
with the inhibition of writhing by a standard analgesic;
indomethacin, given orally at a dose of 20 mg/kg.
Acetic acid (0.7 %) at a dose of 0.1 mL / 10 g was
administered intraperitoneally (i. p.) to create pain
sensation. The number of writhes was calculated for 5
minutes immediately after the acetic acid injection.
The percentage of pain protection was calculated. The
percentage protection of writhing by both test and the
standard drug was calculated according to the following
equation:

\[ \text{Percentage protection} = 100 \times \frac{X_t - X_c}{X_c} \times 100 \]  (8)

Where, \( X_t \) - Average number of writhes in the treated
group, \( X_c \) - Average number of writhes in the control
group.

Formalin-induced rat hind paw oedema test: The
effect of n-hexane extract of \( P. \) pterocarpum stem bark
on formalin-induced inflammation in rat paw was
investigated by following the method described by
(Winter et al., 1962) with minor modifications. Rats
were randomly divided into five groups, each
consisting of five animals. Group I serving as a
negative control was given only distilled water. Group
II, III and IV were given 50, 100 and 200 mg/kg of
the plant extract respectively and Group V, which served
as a positive control was given the standard drug,
aspirin (100 mg/kg.). The animals were fasted 12
hours prior to the experiment. All drugs were
administered orally. Thirty (30) minutes after oral
administration of the test materials, 0.1 mL 1% formalin
suspension was injected subcutaneously in the left hind paw of each animal, leading to the
formation of oedema (localized inflammation) in situ.

The volume of paw oedema was measured hourly for
four hours using a vernier calliper after administration
of formalin. The average percent increase in paw
volume with time was calculated and compared
against the control group. Percent inhibition was
calculated using the formula:

\[ \% \text{Inhibition of paw edema} = 1 - \frac{V_t}{V_c} \times 100 \]  (9)

Where \( V_c \) and \( V_t \) represent average paw volume
of control and treated animal respectively.

Statistical analysis: Data represent mean±SEM and \( n = 5 \) for animals in the group. They were analysed with
one-way analysis of variance (ANOVA), followed by
Bonferroni t-test or student-Newman-Keuls posthoc
tests. \( P < 0.05 \) was considered significant.

RESULTS AND DISCUSSION

Proximate analysis is the determination of a group of
closely associated constituents together (Leslie and
Harry, 2017). Proximate analysis helps to set up a
definite standard for dried crude drugs in order to
avoid batch-to-batch difference and also to judge their
value (Backer and Hyne, 2012). In the present study,
result of proximate composition includes moisture
content (14.53±0.09 %), total ash (7.50±0.10 %), acid
insoluble ash (1.10±0.01 %), water soluble ash (2.00±0.01 %), alcohol soluble extractive value
(28.50±0.50 %) and water-soluble extractive value
(27.00±0.50 %) (Table1). The moisture content
(14.53±0.09 %) of \( P. \) pterocarpum recorded in the
present study was higher than (2.3±0.6 %) reported for
the seed of \( P. \) pterocarpum (Anonymous, 2017)
revealing that the stem bark of \( P. \) pterocarpum is more
susceptible to microbial deterioration than the seed of
the plant. The reason for low moisture content of the
seed could be due to the oil contained in it, which may
act as water repellant. The high total ash is a reflection
of mineral content conserved in the stem bark
(Chinyere et al., 2014). An ash value determination
offers the basis for judging the identity and purity of
any drug and provides information related to its
adulteration with in-organic matters (Falodun et al.,
2013). The total ash of \( P. \) pterocarpum recorded in the
current study was in line with 7.87 % reported by
(Backer and Hyne, 2012). The high total ash of stem
bark of \( P. \) pterocarpum recorded in the present study
was greater than (2.1±0.7 %) reported by (Adewale et
al., 2010) on the seed of \( P. \) pterocarpum. It shows that
stem bark of the plant has higher mineral content than
the seed. Anonymous, 2017 reported total ash of
leaves of \( P. \) pterocarpumas 6.53% indicating that the
stem bark of \( P. \) pterocarpum has higher mineral
content than the leaves. The total ash content was
lesser than the range of 17.44 to 33.60% for mushroom species (Egwim et al., 2011) and higher than the range of 0.38 – 1.9% for certain vegetables grown in Peshawar (Bangash et al., 2011). The acid insoluble ash (1.10±0.01 %) of stem bark of P. pterocarpum recorded in the present study was in line with 1.25 % reported by (Backer and Hyne, 2012). The determination measures the occurrence of silica mainly sand and siliceous earth. The water-soluble ash (2.00±0.01 %) of P. pterocarpum obtained in the present study was in line with 2.2 % recorded in the previous work (Backer and Hyne, 2012). The water-soluble ash was high, showing slight or no adulteration with metal ion (Falodun et al., 2013). The alcohol soluble extractive value (28.50±0.50 %) of P. pterocarpum obtained in this study is similar to the result (30 %) obtained by Backer and Hyne, 2012. It determines the number of active components extracted with alcohol from a given amount of plant drug. Alcohol is a better solvent for extraction of phytoconstituents from the stem bark of P. pterocarpum because more phytoconstituents were extracted in it. The water-soluble extractive value (27.00±0.50 %) of P. pterocarpum recorded in the present study is also similar to the result (28 %) obtained by Backer and Hyne, 2012. It determines the number of active components extracted with aqueous solvent from a given quantity of plant drug (Table 1).

The result for the acute toxicity in which the LD₅₀ obtained was 158.11mg/kg was in line with existing literature by (Syed et al., 2012) who documented an LD₅₀ value of 122.47 mg/kg for the leaves of the plant. In inspecting drugs, determination of LD₅₀ is one of the first stages in evaluating the lethal nature of a material (Ogbeide et al., 2018; Ogu et al., 2012). In this study, there was no indication of toxicity and variation in behavioural pattern observed in the experimental animals treated with the extract for doses 5 mg/kg and 50 mg/kg. However, at doses 500 and 2500 mg/kg, there were observable signs of toxicity such as restlessness, refusal of feed, seizures and death. According to Hodge and Sterner scale which proposes that, a test drug administered orally is considered extremely toxic at ≤ 1 mg/kg, highly toxic at 1-50 mg/kg, moderately toxic at 50-500 mg/kg, slightly toxic at 500-5000 mg/kg, practically non-toxic at 5000-15,000 mg/kg and relatively harmless at ≥15,000 mg/kg (Ogbeide et al., 2018). Hence, from the present study, P. pterocarpum stem bark could be moderately toxic (Table 2).

### Table 2: Acute toxicity of the n-hexane extract of P. pterocarpum stem bark

<table>
<thead>
<tr>
<th>Groups</th>
<th>Doses (mg/kg)</th>
<th>Mortality</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.p extract</td>
<td>5</td>
<td>0/3</td>
<td>0</td>
</tr>
<tr>
<td>P.p extract</td>
<td>50</td>
<td>0/3</td>
<td>0</td>
</tr>
<tr>
<td>P.p extract</td>
<td>500</td>
<td>1/3</td>
<td>33</td>
</tr>
<tr>
<td>P.p extract</td>
<td>2500</td>
<td>2/3</td>
<td>66</td>
</tr>
<tr>
<td>Control (Tw)</td>
<td>0.2 MI</td>
<td>0/3</td>
<td>0</td>
</tr>
<tr>
<td><strong>P.p: P. pterocarpum stem bark; Tw: Tween 80 (control); LD₅₀ = (highest non-lethal dose × lowest lethal dose)⁻¹; LD₅₀ = 158.114mg/kg</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analgesic or pain killer is any member of the set of drugs used to attain analgesia, free from pain. Analgesic drugs act in several ways on the peripheral and central nervous systems. In the acetic acid-induced writhing, n-hexane extract of the stem bark of P. pterocarpum, significantly (p<0.05) showed a dose-dependent decrease in the number of writhes within 5 minutes of injection of acetic acid (0.7 %/w/v) when related to control. The n-hexane extract of P. pterocarpum (100 and 200 mg/kg) showed a significant reduction in a number of writhes with 55.6 and 60% of inhibition respectively (see Table 3). The plant extract at a dosage of 50 mg/kg showed 0 % inhibition. Maximum inhibition of writhing was observed at 200 mg/kg with 60 % compared with standard indomethacin (76.5 %). The extract protected the mice against chemically induced noxious stimuli (Sarvesh et al., 2017). Intra-peritoneal administered acetic acid produced high levels of prostaglandins PGE2 and PG-F₂α in peritoneal fluid (Sarvesh et al., 2017). The abdominal constriction in mice was related to sensitization of peritoneal nociceptors by prostaglandin (Bose et al., 2007; Sengar et al., 2015). The abdominal contraction response induced by acetic acid is a sensitive technique to establish peripherally acting painkillers (Achinta et al., 2007). The analgesic effect displayed by the extract is connected to the inhibition of prostaglandin level (Sarvesh et al., 2017) (Table 3). Inflammation induced by formalin caused the formation of rat paw oedema. In the negative control group, the size of the paw continued to rise with time until after two hours when it began to fall. This decrease in paw size of the negative control group is probably due to the action of antibodies combating inflammation in the animal. In the reference and test groups, however, there was noticeable rise in the paw size within hours, due to the action of the antioxidants existing in them (Table 4).

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The results of the present study showed the dosage-dependent activity of the plant extract in hindering inflammation with % inhibition of 26.00 at 30 mg/kg (least dosage) and 32.27 at 200 mg/kg (highest dosage) after 4 hours. The % inhibition of the reference drug, aspirin, was 33.59; hence the plant exhibited activity comparable to that of the standard medicine. Another study described by (Payal, 2012) of the stem bark of methanol extract, documented % inhibition of 13.02 at 300 mg/kg after five hours. This difference in the result may be due to the use of dissimilar solvents. However, other influences that may affect the results include the season, time of harvest, age and maturity of plant, place where the sample was obtained and other environmental factors (Ogbeide et al., 2018) (Table 5).

Table 5: % inhibition of P. pterocarpum stem bark

<table>
<thead>
<tr>
<th>Group</th>
<th>% inhibition</th>
<th>1 hr</th>
<th>2 hrs</th>
<th>3 hrs</th>
<th>4 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Dw)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ref. drug</td>
<td>33.59</td>
<td>33.59</td>
<td>33.59</td>
<td>33.59</td>
<td>33.59</td>
</tr>
<tr>
<td>P.p extract</td>
<td>27.67</td>
<td>27.67</td>
<td>27.67</td>
<td>27.67</td>
<td>27.67</td>
</tr>
<tr>
<td>P. p extract</td>
<td>32.41</td>
<td>32.41</td>
<td>32.41</td>
<td>32.41</td>
<td>32.41</td>
</tr>
<tr>
<td>P. p extract</td>
<td>33.59</td>
<td>33.59</td>
<td>33.59</td>
<td>33.59</td>
<td>33.59</td>
</tr>
</tbody>
</table>

Conclusion: This study established that the n-hexane extract of stem bark of P. pterocarpum could be a promising source of analgesic and anti-inflammatory agent. To the best of our knowledge, this is the first report on the analgesic and anti-inflammatory activities of n-hexane extract of P. pterocarpum stem bark. Further phytochemical studies are ongoing in our laboratory to isolate the bioactive principles responsible for the analgesic and anti-inflammatory activities.

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