AN EVALUATION OF THE ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF THE SOLVENT FRACTIONS OF 

ASPILIA AFRICANA (PERS.)

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ABSTRACT

The use of herbal medicines in the treatment of pain and inflammation has increased significantly in recent years as herbal drugs have become the most accessible form of healthcare to majority of the population in most developing nations. This study was therefore aimed at the investigation and evaluation of the analgesic and anti inflammatory effects of the solvent fractions of Aspilia africana on tail-immersion-induced pain and egg-albumin-induced inflammation using albino rats. Phytochemical constituents of the solvent fractions were also determined using standard procedures. Saponins, tannins and flavonoids were found to be common in all the active fractions. The analgesic and anti-inflammatory effects of the different solvent fractions were compared against a standard non-steroidal anti-inflammatory drug (Indomethacin) and a placebo. All the fractions showed negative significant values at P<0.05 when compared with Indomethacin for the analgesic effect while that against the placebo showed no significant difference in activity. For the anti-inflammatory effect, all the fractions showed no significant difference when compared with indomethacin but when compare with the negative control, a positive significant difference (P < 0.001) was observed from the 3rd to 4th hours. In conclusion, Aspilia africana leaves harvested in Nigeria could therefore be said to posses significant anti inflammatory property with little analgesic effect. This makes the plant a
useful agent in the formulation of herbal-based preparations that could be employed in combating numerous cases of arthritic and other inflammatory disorders common among the people leaving in that region.

**Keywords:** Analgesic; Anti-inflammatory; Arthritis; *Aspilia*; Fractionation; Indomethacin.

### 1.0 INTRODUCTION

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. It can also be described as a complex reaction to injurious agents such as microbes and damaged, usually necrotic, cells that consist of vascular responses, migration and activation of leukocytes and systemic reactions.\(^1\) It can be evoked by a wide variety of noxious agents (e.g., infections, antibodies or physical injuries). Although the ability to mount an inflammatory response has been described as essential for survival in the face of environmental pathogens and injury; but in some situations and diseases, the inflammatory response may be exaggerated and sustained without apparent benefit and even with severe adverse consequences.\(^2\)

Inflammatory abnormalities are a large group of disorders which underlie a vast variety of human diseases. The immune system is often involved with inflammatory disorders, demonstrated in both allergic reactions and some myopathies, with many immune system disorders resulting in abnormal inflammation. Non-immune diseases with etiological origins in inflammatory processes include cancer, atherosclerosis, and ischaemic heart disease.\(^3\) Arthritis is classified as a rheumatic disease, a type of disease that affects joints, muscles, ligaments, cartilage and tendons; frequently causing joint destruction and functional disability.\(^4\) It is characterized by inflammation of the joints which is frequently accompanied by joint pain, referred to as arthralgia. Treatment of patients with inflammatory disorders usually involves the relief of pain (which is often the presenting symptom and the major continuing complaint of the patient) and the slowing down or arrest of the tissue-damaging process.\(^5\) Non steroidal anti inflammatory drugs (NSAIDs) are usually indicated for the treatment of acute or chronic conditions where pain and inflammation are present.\(^6\) All the steroidal and NSAIDs, despite their usefulness, cause undesired and serious side effects which in many cases are severe enough to pose the risk of ulcer perforation, upper gastrointestinal bleeding, and death, limiting their use in therapy.\(^7\) Therefore, development of new and more powerful drugs (e.g. purified natural compounds from plants which can
serve as template for the synthesis of new generation anti-inflammatory drugs) with low toxicity and higher therapeutic value is still needed.

Medicinal plants have long been used worldwide in folk medicine as an alternative treatment of inflammatory processes of diverse origins. Some of the medicinal plants that have been investigated for their analgesic, anti-inflammatory or anti-rheumatic activities include: Cannabis (*Cannabis sativa L.*), Pineapple (*Ananas comosus L.*), Green tea (*Camelia sinensis L.*), Mango (*Magnifera indica L.*), Clove (*Syzygiumaromaticum*), Ginger (*Zingiber officinale R.*), Elderberry (*Sambucus nigra*), Eucalyptus (*Eucalyptus globulus*), European Mistletoe (*Viscum album*), Evening primrose (*Oenothera spp.*), Banyan (*Ficus bengalensis*), etc. [9-12] *Aspillia africana* (Asteraceae) is a tropical shrub widely grown in Nigeria, where it is commonly known as *yurinyun* by the Yorubas, *orangila* by Igbos, *tozalin* by Hausas and *Edemedong* by Efiks. [13] It is a perennial herb varying in height from 60cm to about 1.5m depending on rainfall. The plant is a common weed grazed by cattle and sheep and is mostly used in the southern part of Nigeria as food for rabbits and hares. [14] It is widely used in ethnomedical practice in Africa for its ability to stop bleeding, even from a severed artery, as well as promote rapid healing of wounds and sores and for the management of problems related to cardiovascular diseases. [15] *Aspilia* is also used in traditional medicine for the treatment of several ailments in different parts of the world. Such ailments include: gonorrhea, tuberculosis, cough, rheumatic pains, stomach trouble, corneal opacity, wounds and insect bites. [13] It has been classified among substances with low toxicity, with an LD$_{50}$ averaging 6.6g/Kg body weight. [16] Some of the pharmacological activities of the plant that have been investigated are antimalarial, [17] anti-inflammatory, [18] anticoagulant, [19] antimicrobial, [17,20] wound healing, [15,18] contraceptive and anti-fertility [21] activities.

Several medicinal plants have been used in traditional practice across Africa to combat painful and rheumatic disorders prevalent in that region. Currently, many of these herbal remedies are available in Nigerian and other West African markets with bogus claims of efficacy, hence the need for scientific validation of these claims and establishment of the mechanisms behind their activities. This research work was, therefore, aimed at evaluating the phytochemical constitution, analgesic and anti-inflammatory effects of four solvent fractions of the leaves of *Aspilia africana* growing in the tropical rainforest region of Nigeria, Sub-Saharan Africa.
2.0 MATERIALS AND METHODS

2.1 Plant Material
The leaves of the plant (*Aspilia africana*) were collected from the Botanical Reserved Area within the University of Port Harcourt in July, 2012. It was authenticated by Mrs. Margaret Bassey, a taxonomist and an Associate Professor in the Department of Botany and Ecological studies, University of Uyo, Nigeria. A voucher specimen of the plant was deposited in the Pharmacognosy Herbarium of the University of Port Harcourt. The leaves were prepared by drying under shade for 14 days. The dried leaves were pulverized and stored in air tight containers kept inside the refrigerator.

2.2 Animals Used
Thirty (30) albino rats consisting of both sexes and weighing between 150 and 225g were obtained from the University of Port Harcourt Animal House, quarantined and acclimatized for 10 days before treatment in a standard environment. The animals were fed with standard feed and water *ad libitum*. All the standard ethical requirements for experimental animals were complied with.

2.3 Reagents Used
Analytical grade solvents such as methanol, dichloromethane, n-hexane, ethylacetate and butanol (all sourced from SIGMA-ALDRIVIR, Germany) were used. Freshly prepared laboratory standard reagents were also employed.

2.4 Extraction of the Plant Material
Three hundred and fifty gram (350g) of the pulverized dried leaves of the plant was macerated with 70% v/v methanol (1.5L) at room temperature. Three (3) different filtrates were collected at intervals of 72 hours. These filtrates were later combined and concentrated *in vacuo* using a rotary evaporator.

2.5 Fractionation Method
The crude hydro-alcoholic concentrate from the above extract was made completely free of the alcohols by leaving it to evaporate at room temperature thereby obtaining the aqueous suspension (150ml) of the crude methanol extract. The sequential partitioning was done using n-hexane, dichloromethane, ethyl acetate, and n-butanol. The process was carried out using 1L globe-shaped separating funnels starting with n-hexane (3 X 350ml), dichloromethane (3 X 350ml), ethyl acetate (3 X 350ml), and finally n-butanol (3 X 350ml) to yield the n-hexane,
dichloromethane, ethyl acetate and butanol fractions respectively. Similar solvent fractions were combined, concentrated in vacuo and air-dried. They were weighed, stored in air-tight containers and preserved in the refrigerator for subsequent use.

2.6 Phytochemical Tests
Phytochemical tests were carried out according to the procedures outlined by Harborne\cite{22} to detect the presence of steroids, alkaloids, tannins, glycosides, reducing sugars, flavonoids and saponins.

2.7 EVALUATION OF ANALGESIC ACTIVITY
2.7.1 Tail immersion test
The test was based on the method as described by Ramabadran et al.\cite{23} Thirty (30) albino rats (150-225) g were divided into six (6) groups of five (5) animals each, and placed in six (6) cages labeled A-F accordingly. The animals were allowed to acclimatize for 76 hours. Animals in groups A-D received 200mg/kg of dichloromethane, n-hexane, butanol and ethyl acetate extracts respectively, group E received 10mg/kg of the standard non steroidal anti inflammatory drug, Indomethacin serving as the positive control group and group F received only distilled water serving as the negative control group orally. The distal part of the tails of the animals was immersed in hot water maintained at 55.0 ± 1.0°C. The time taken to withdraw the tail was noted as reaction time. A cut off time of 10 seconds was maintained at 55°C to prevent tissue damage. The reaction time was measured at 0, 15, 30, 45 and 60 minutes after treatment respectively.

2.8 EVALUATION OF ANTI INFLAMMATORY ACTIVITY
2.8.1 Egg albumin induced paw edema
The test was based on the method as adopted by Winter et al.\cite{24} In this case, egg albumin served as the edematogenic agent. Acute inflammation was measured in terms of change in volume of the rat hind paw induced by sub plantar injection of egg albumin. Animals in groups A-D received 200mg/kg of dichloromethane, n-hexane, butanol and ethyl acetate extracts respectively, group E received 10mg/kg of indomethacin (serving as the positive control group) and group F received distilled water (serving as the negative control group) orally. Edema was induced one (1) hour later with 0.1ml egg albumin injected into the subplantar region of the right hind paw of the rats. The course of the edema was monitored by measuring the thickness of footpad swelling before and at 1, 2, 3, and 4 hours after egg albumin injection by using a vernier caliper.
The percentage inhibition of inflammation was calculated using the formula:

\[
\% \text{ Inhibition} = \left( \frac{tC_0 - tC_n - tT_n + tT_0}{tC_0 - tC_0} \right) \times 100
\]

Where;

- \( tC_n \) = Paw thickness at a particular time for control animal
- \( tC_0 \) = Paw thickness before induction for control animal
- \( tT_n \) = Paw thickness at a particular time for treated animal
- \( tT_0 \) = Paw thickness before induction for treated animal

3.0 STATISTICAL ANALYSIS

All the data obtained were analyzed by GraphPad Prism® (Model 5) using two-way ANOVA and subjected to Bonferroni post-tests to compare replicate means. The statistical results were presented as Mean ± SEM. Differences between means were considered significant at \( P<0.05 \).

4.0 RESULTS AND DISCUSSION

A two(2) way analysis of variance(ANOVA) done in order to compare the analgesic effect of the solvent extracts of \textit{Aspilia africana} with a standard non steroidal anti inflammatory drug (indomethacin) showed mostly negative significant values at \( P < 0.05 \) while the comparison against the negative control showed no statistically significant values (Table 1).

**Table 1: Analysis of the analgesic effect of fractions of \textit{Aspilia africana} using the tail immersion method against standard (Indomethacin)**

<table>
<thead>
<tr>
<th></th>
<th>0 mins</th>
<th>15 mins</th>
<th>30 mins</th>
<th>45 mins</th>
<th>60 mins</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-hexane extract</td>
<td>2.60±0.40</td>
<td>2.40±0.25</td>
<td>3.20±0.20</td>
<td>2.60±0.25</td>
<td>2.60±0.25*</td>
</tr>
<tr>
<td>Dichloromethane extract</td>
<td>2.60±0.40</td>
<td>2.20±0.20*</td>
<td>2.40±0.25</td>
<td>2.80±0.37</td>
<td>2.80±0.37</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>3.60±0.25</td>
<td>2.20±0.20*</td>
<td>2.40±0.40</td>
<td>2.80±0.50</td>
<td>3.00±0.32</td>
</tr>
<tr>
<td>Butanol fraction</td>
<td>2.40±0.68*</td>
<td>2.60±0.40</td>
<td>3.60±0.51</td>
<td>3.00±0.45</td>
<td>3.40±0.51</td>
</tr>
<tr>
<td>Distilled Water (-ve Control)</td>
<td>3.80±0.86</td>
<td>2.80±0.49</td>
<td>2.60±0.25</td>
<td>2.40±0.25*</td>
<td>2.40±0.25*</td>
</tr>
<tr>
<td>Indomethacin (+ve Control)</td>
<td>2.20±0.58</td>
<td>4.00±0.71</td>
<td>3.00±0.58</td>
<td>2.40±0.40</td>
<td>2.80±0.58</td>
</tr>
</tbody>
</table>

Key: Values presented in the table represent mean ± SEM reaction time in seconds; \( n=5 \).

*= \( P<0.05 \) statistically significant values when compared with positive control group.
The data in Table 1 show that the mean ± SEM values of the reaction time for analgesic effect recorded for each extract decreased as the number of minutes of exposure increased, which suggests that there was no analgesic effect at that time (an increase in the reaction time with time shows analgesic activity). This result suggests that the solvent fractions of *Aspilia africana* had little or no analgesic effect compared with indomethacin in this test. This observation could be as a result of a longer onset of action by the extracts as compared with indomethacin or a complete lack of analgesic effect by the plant.

The anti-inflammatory evaluation results of the solvent fractions of *Aspilia africana* showed no significant difference when compared with indomethacin while the comparison against the negative control group showed a positive significant difference up to P < 0.001 (Table 2).

**Table 2: Analysis of the anti-inflammatory effect of fractions of *Aspilia africana* on egg albumin induced paw edema against the control with mean % decrease in paw edema**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>0 hour</th>
<th>1 hour</th>
<th>2 hours</th>
<th>3 hours</th>
<th>4 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-hexane fraction</td>
<td>7.20±0.37</td>
<td>8.60±0.25</td>
<td>8.20±0.20</td>
<td>8.00±0.00***</td>
<td>8.00±0.32***</td>
</tr>
<tr>
<td>Dichloromethane Fraction</td>
<td>7.60±0.29</td>
<td>9.80±0.20*</td>
<td>9.10±0.25 (21.1%)</td>
<td>8.40±0.25** (69.2%)</td>
<td>8.00±0.00*** (84.6%)</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>7.60±0.30</td>
<td>9.30±0.37 (5.6%)</td>
<td>9.20±0.34 (5.6%)</td>
<td>8.80±0.37 (53.9%)</td>
<td>8.00±0.00*** (84.6%)</td>
</tr>
<tr>
<td>Butanol fraction</td>
<td>7.60±0.25</td>
<td>9.60±0.40 (0%)</td>
<td>8.20±0.12 (64.86%)</td>
<td>8.00±0.32*** (84.6%)</td>
<td>7.70±0.20*** (96.2%)</td>
</tr>
<tr>
<td>Indomethacin (+ve Control)</td>
<td>7.00±0.00</td>
<td>9.40±0.25 (0%)</td>
<td>8.50±0.22 (21.1%)</td>
<td>8.30±0.20** (50%)</td>
<td>7.90±0.33*** (65.4%)</td>
</tr>
<tr>
<td>Pure solvent (-ve Control)</td>
<td>7.00±0.00</td>
<td>8.80±0.37</td>
<td>8.90±0.10</td>
<td>9.60±0.25</td>
<td>9.60±0.40</td>
</tr>
</tbody>
</table>

Key: Values presented in the table represent mean ± SEM paw diameter in mm; n=5. Numbers in parenthesis indicate mean percentage decrease in paw edema; *= P<0.05, **=P<0.01 and ***=P<0.001 statistically significant values when compared with negative control group.

The table also shows that the mean ± SEM values of the diameter of paw edema decreased as the number of hours of exposure increased while percentage inhibition values increased.
Among the fractions, the n-hexane and butanol fractions showed significant values at $P < 0.001$ by the third (3rd) hour, while dichloromethane fraction showed a significant value of $P < 0.01$ at the same period (same as indomethacin). By the fourth (4th) hour, all the fractions showed significant values at $P < 0.001$ including the standard, indomethacin. This clearly indicates that their onset of action may be within the range of three (3) hours. The hexane and butanol fractions exhibited the fastest onset of action. This late onset of action (3 hours) could equally have been responsible for the poor analgesic effect observed within the 60 minutes the analgesic evaluation was carried out.

Carrageenan (or egg albumin)-induced acute inflammation is one of the most suitable test procedures to screen anti-inflammatory agents. The time course of edema development using this model in rats is generally represented by a biphasic curve.\textsuperscript{[25]} The first phase of inflammation occurs within an hour of the edematogenic agent injection and is partly due to the trauma of injection and also due to histamine and serotonin component.\textsuperscript{[26]} There was no significant inhibition of paw edema in the early hours of the study by the solvent extracts of \textit{Aspilia africana}. Hence, it can be concluded that there is no inhibition of histamine and serotonin. Carrageenan (egg albumin)-induced paw edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents, which primarily inhibit the cyclooxygenase involved in prostaglandin synthesis.\textsuperscript{[27]} It plays a major role in the development of the second phase of inflammatory reaction, which is measured at the 3rd hour.\textsuperscript{[28]} As shown in the result, there is a significant ($P < 0.01$ and $P < 0.001$) percentage inhibition of paw edema at the 3rd hour by the solvent extracts. Therefore, it can be inferred that the inhibitory effect of the solvent fractions on egg albumin-induced inflammation may be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis. These tend to suggest that the anti-inflammatory effects of the solvent fractions of \textit{Aspilia africana} follow the same mechanisms of pharmacological actions as indomethacin on pain and inflammation with almost the same onset of action.

The phytochemical study carried out on the solvent fractions of \textit{Aspilia africana} revealed that carbohydrates, saponins, tannins/phenolic compounds and flavonoids were evenly distributed among the fractions (Table 3).
Table 3: Phytochemical profile of the fractions of *Aspilia africana*

<table>
<thead>
<tr>
<th></th>
<th>N-hexane fraction</th>
<th>Dichloromethane fraction</th>
<th>Ethyl acetate fraction</th>
<th>Butanol fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinone glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Reducing Sugars</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Key:* + present; - = Absent

Flavonoid which was dominant in the test carried out is a major anti inflammatory agent. Flavonoids act as phospholipase inhibitors and some have demonstrated to be tumor necrosis factor inhibitors in different inflammatory conditions. Biochemical investigations have also shown that flavonoids can inhibit both cyclooxygenase and lipoxygenase pathways of the arachidonic metabolism depending upon their chemical structures.[29,30] Saponin is an antioxidant and there are well-known interactions between rheumatoid arthritis, chronic inflammatory disease, food and nutrition.[31,32] Of particular importance are nutrients that stimulate the formation of oxidants and peroxides (e.g. unsaturated fatty acids, iron), which promote inflammatory disease, and antioxidants (e.g. vitamin E) and omega-3 fatty acids, which protect against auto-oxidation. Platelet aggregation is also characteristic of inflammation. Yucca phenolics have been shown to possess inhibitory activity against platelet aggregation.[33,34,35] Blood platelets participate in allergic inflammation responses.[36]

5.0 CONCLUSION

*Aspilia africana* is one of the medicinal plants used traditionally for various painful and inflammatory disorders in Nigeria and other parts of sub-Saharan Africa. The present study suggests that the plant has some clinical benefits and is a potential alternative for the management of inflammation and consequently rheumatoid arthritis in areas where the first
line treatments of choice are not easily accessible or affordable. The presence of phytoconstituents like flavonoids, tannins, and saponins may be partly or wholly responsible for its anti-rheumatic/anti-inflammatory activity as well as the modification of the autoimmune system. Also, the acute toxicity test on the plant has indicated a wide therapeutic margin. Based on these observations and results, there is clearly a justification for the ethnomedicinal and folkoric use of *Aspilia africana* as an anti-inflammatory or anti-rheumatoid agent in Nigeria and other parts of Africa.

**AUTHORS’ STATEMENT**

**Competing Interests**
The authors declare no conflict of interest.

**REFERENCES**


