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# Phytochemistry and Anti-Inflammatory Activities of *Aspilia africana* (Pers) C.D. Adams

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**Abstract**
Qualitative/quantitative phytochemical screening and anti-inflammatory activity of *Aspilia africana* was investigated using the carrageenan. Carrageenan-induced rat paw edema is a widely used test to determine anti-inflammatory activity and constitutes a simple and routine animal model for evaluation of pain at the site of inflammation without any injury or damage to the inflamed paw. Qualitative and quantitative phytochemical screening, Acute Toxicity Test ($LD_{50}$) and inflammatory analysis were carried out. Results revealed *A. africana* contained saponins, tannins, combined anthraquinone, cardiac glycocides, terpenes and flavonoids in appreciable amounts. The mice were treated intraperitoneally with a single dose of 0.09 – 0.64 mg/kg of *A. africana* ethanolic leave extracts after being left without food for 18 hours. Intraperitoneal route was chosen because of its sensitivity and rapid results. These ethanolic leaf extracts produced various degree of toxicity ranging from writhing, decreased respiration to mortality. The intensities of these effects were proportional to the dose administered. The intraperitoneal $LD_{50}$ for *A. africana* was 489.99mg/kg. Toxicity analysis revealed that the leaves *A. africana* are non toxic at a particular dose. Administration of different percentages of ethanolic leaf extracts showed marked inhibition at different measured interval and extract of *A. africana* prevented the formation of edema induced by carrageenan, thus showing significant anti-inflammatory activity ($p=0.05$) and also inhibited the edema by injection of noxious agent as compared to the control group. Also, ethanolic leaf extracts of leaves *A. africana* inhibited inflammation of hind paw edema of albino rats induced by carrageenan. The synergistic effect of saponin and flavonoid present in the plant which has been investigated to possess anti inflammatory activity is suggested to have caused rapid inhibition of the edema of the hind paw induced by carrageenan. Therefore, the efficacy and efficiency of leaves of *A. africana* is recommended therapeutically, locally, traditionally and pharmaceutically for inhibition and treatment of inflammation.

**Keywords:** acute toxicity, albino rats, *Aspilia africana*, carrageenan, inflammation phytochemicals

## Introduction
The use of herbs to treat disease is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceuticals [1]. World health organization [1] estimates that 80 percent of the population of some Asian and African countries presently uses herbal medicine for some aspect of primary health care. Studies in United States and Europe have shown that their use is less common in clinical settings, but has become increasingly more common in recent years as scientific evidence about the effectiveness of herbal medicine has become more widely available. The annual global export value of pharmaceutical plants in 2011 accounted for over US$2.2 billion [1]. Medicinal plants are the richest bio-resource for drugs in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [2].

*Aspilia africana* commonly called haemorrhage or wild marigold plant originated from west tropical Africa, an angiosperm of Asteraceae (Compositae) family, is a semi woody herb from the perennial woody root stock, up to 2 m high, a very polymorphic with at least four varieties recognized throughout the region on wasteland of the savanna and forested zones and widely distributed across tropical Africa. Leaf and flower are mostly useful medicinally. Extracts of leaves of *Aspilia africana* have good potential for use in wound care and wound management [3]. A study showed that *Aspilia africana* could be used in the treatment of anaemia in rabbits when fed as forage [4]. *Aspilia africana* enhanced gastroprotection via reduction of acid output, neutrophil infiltration and oxidative stress as reported by Ajeigbe *et al.* [5]. The findings of antimalarial potency of ethanol extract of *A. africana* leaf shows that it possesses potent antiplasmodial activity which justify the use in ethnomedicine and can be developed in malarial therapy [6].

Inflammation is described as "the succession of changes which occur in a living tissue when it is injured provided that the injury is not of such a degree as to at once destroy its structure and vitality" [7]. The classical signs of acute inflammation are tumor (swelling of the tissue), calor (elevated tissue temperature), rubor (redness of vascularized tissue at inflammation site), dolor (intensive sensation of a noxious stimulus), and function laesa (impaired functioning of the affected organ) [8].

**Fig 1:** Leaves of *Aspilia africana* (Pers) C.D. Adams.

Inflammation is a generic response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen [9]. Thus, this research was carried out to evaluate the phytochemical constituents and toxicity level ($LD_{50}$) of *Aspilia africana* (leaves) extract on the experimental mice.

## Materials and Methods

### Plants Collection and Authentication

The leaves of *Aspilia africana* were collected from plants growing in the Department of Botany and Ecological Studies Botanic Garden, University of Uyo, Akwa Ibom State, Nigeria. The plant samples were authenticated by a Plant Taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria.

### Preparation of Plant Extracts

The fresh leaves of *A. africana* were chopped into small pieces and air dried for 7 days and made into a coarse powdered form, 400 g of the powdered sample was extracted using 6000 ml of 70% ethanol and shaken intermittently for 72 hours. It was filtered and the filtrate concentrated (dried) *in-vacuo* at 40˚C in a water bath.

### Qualitative and Quantitative Phytochemical Screening

The methods described by [10 – 12] were used for qualitative and quantitative phytochemical screening of *A. africana* leaf extract. These included tests for saponins, tannins, flavonoids, anthraquinone, terpenes, phlobatannins alkaloids and cardiac glycosides. The experiment was carried out in the Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo.

### Acute Toxicity Test ($LD_{50}$ Determination)

Fifty Swiss Albino Mice (*Mus musculus*) weighing 18 – 35g were used to determine the $LD_{50}$ of the extract. Five groups of five mice each were kept and handled according to standard guidelines for the use and care of laboratory animals. Food was withdrawn for 18 hours before the onset of the experiment according to methods of [13]. The mice were administered with 200-1000 mg/kg of *Aspilia africana*. The groups were observed for manifestation of physical signs of toxicity and mortality rate within 24 hours, the median lethal dose ($LD_{50}$) was calculated for intraperitoneal route (i.p.) of administration according to the methods of [14] with this formula:

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

Where: $D_0$ = Maximum dose producing 0% mortality
$D_{100}$ = Minimum dose producing 100% mortality

### Experimental Animals

Fifty either sex albino rats weighing 80 – 120g were randomly assigned to five groups of five rats in each group and were kept at the Laboratory Animal Unit, Faculty of Pharmacy, Department of Pharmacology and Toxicology Laboratory, University of Uyo Nigeria for assessment of inflammatory activities. The 10%, 20% and 30% of $LD_{50}$ were used as working doses implying low, middle and high dose.
Group I received 10 ml/kg of normal saline,
Group II received 100 mg/kg of standard drug (Aspirin),
Group III received 48.99 mg/kg of *Aspilia africana* extract,
Group IV received 97.98 mg/kg of *Aspilia africana* extract,
Group V receiveD 146.92 mg/kg of *Aspilia africana* extract.

### Preparation of Extract and Fraction Solutions

The extract and fraction solutions were prepared by dissolving about 1.0g (using high performance profile design compact weighing balance: CS200) of the extract and fractions in 10 ml of distilled water, to give an effective concentration. The solutions were prepared fresh daily before administration [2]. The formula below was used to calculate the volume of the extract/fractions solution administered to each animal.

$$WSF = \frac{WR}{1000} X \frac{EM}{STOCK}$$

Where; WSF = Water Soluble Fraction
WR = Weight of Rat
EM = Estimated milligram/kilogram of plant extract

### Inflammatory Analysis

Inflammation was examined using Carrageenan-induced paw oedema in rats according to the method of Winter *et al*. [15] with slight modifications. Five groups of five rats each were treated orally with dose of 48.99, 97.98 and 146.97 mg/kg of *Aspilia africana* per body weight, ASA (Standard Drug - 100 mg/kg) and normal saline (10 ml/kg). One hour after the administration of the various agents, edema was induced by injecting Carrageenan (0.1ml, 1% in saline) into the subplantar tissue of the right hind paw. Paw edema was measured with a vernier calliper. Measurements were made immediately before injection of the phlogistic agents and afterwards at 1 h intervals, for 5 h.
The percentage inhibition was calculated thus:

$$\% \, Inhibition = \frac{V_c - V_t}{V_c} \; X \; 100$$

Where: $V_c$ = Mean increase in paw circumference in control group,
Vt = Mean increase in paw circumference in test group

**Statistical Analysis**

One way analysis of variance was adopted for comparison of means to find out if there was any significant difference between the control with the treated groups and the results were expressed as mean ± standard error of mean (S.E.M.) of five replicates.

**Results and Discussion**

Qualitative phytochemical screening of ethanolic leaf extracts of *Aspilia africana* revealed a number of bioactive constituents, as summarized in Table 1. Ethanolic leaf extracts of *Aspilia africana* revealed alkaloids were absent; saponins, tannins, combined anthraquinone and cardiac glycocides were moderately detected. Terpenes and flavonoids were detected in trace amount (Table 1).

**Table 1:** Qualitative phytochemical screening of *Aspilia africana* leaves.

| Constituent | Test | Observation | *Aspilia africana* |
|---|---|---|---|
| Alkaloids | Dragendoff's | Formation of red precipitate indicated the presence of alkaloids. | - |
| Tannins | Ferric Chloride | a blue-green precipitation | ++ |
| Saponins | Frothing | Formation of 1 cm layer of foam was observed. | ++ |
| Terpenes | Libermanns Burchards | formation of brown ring at the junction indicated the presence of phytosterols | + |
| Flavonoids | Shinoda | a red colour indicated the presence of flavonoids | + |
| Phlobatannins | | no visible colour | - |
| Free Anthraquinone | Borntrager's | the presence of a pink in the ammoniacal (lower) phase | + |
| Combined Anthraquinone | Sulphuric acid | violet coloration in the ammonia phase (lower layer) | ++ |
| Cardiac Glycosides | Salkowski | a reddish-brown colour at the interface | + |
| | Keller-kellani | brown ring obtained at the interface | ++ |

+ Tracely Detected, ++ moderately detected, +++ abundantly detected, - Not detected

Quantitative phytochemical screening of ethanolic leaf extracts of *Aspilia africana* revealed a number of bioactive constituents such as alkaloids, flavonoids, tannins, saponins and cardiac glycosides were quantitatively estimated and the values mean (±SEM) are presented in Table 2.

**Table 2:** Quantitative Phytochemical Screening of leaf extracts of *Aspilia Africana*

| Constituents | *Aspilia africana* (%) |
|---|---|
| Alkaloids | 0.00 ± 0.00 |
| Tannins | 6.64 ± 0.42 |
| Saponins | 2.27 ± 0.15 |
| Cardiac glycocides | 0.70 ± 0.09 |
| Flavonoids | 2.10 ± 1.43 |

Data are displayed as mean (±SEM) of triplicate experiments

**Toxicity of *Aspilia africana* Ethanolic Leaves Extract**

The mice were treated intraperitoneally with a single dose of 0.09 – 0.64 mg/kg of *Aspilia africana* ethanolic leave extracts after being left without food for 18 hours. Intraperitoneal route was chosen because of its sensitivity and rapid results. These ethanolic leaf extracts produced various degree of toxicity ranging from writhing, decreased respiration to mortality. The intensities of these effects were proportional to the dose administered. The intraperitoneal for *Aspilia africana* it was 489.99mg/kg (Table 3).

**Table 3:** Toxicity of *Aspilia africana* leaf extracts on Swiss Albino Mice (*Mus musculus*).

| Groups | Dose (mg/kg) | Wt. of mice (g) | No. of mice per group | % mortality |
|---|---|---|---|---|
| I | 1000 | 24 | 5 | 100 |
| II | 800 | 25 | 5 | 100 |
| III | 600 | 26 | 5 | 40 |
| IV | 400 | 24 | 5 | 0 |
| V | 200 | 25 | 5 | 0 |

Where: 100 = Maximum dose producing 100% mortality
0 = Minimum dose producing 0% mortality
Wt = Weight

**Anti-Inflammatory Activities of Ethanolic Leaf Extracts of *Aspilia africana***

The effects of oral administration of ethanolic leaf extracts of *Aspilia africana* (48.99, 97.98 and 146.97 mg/kg b.w.) in carrageenan induced paw edema in albino rats is shown in Table 4. The extract of *Aspilia africana* (146.97 mg/kg) obviated the formation of edema induced by carrageenan and thus showed significant anti-inflammatory activity (p=0.05). Also, the paw size was reduced from $6.75^{\pm}0.09$ mm to $5.70^{\pm}0.16$ mm (Table 4). The ethanolic extract of *Aspilia africana* (146.97 mg/kg) receded the edema induced by carrageenan by 17.03% after 5h of injection of phlogistic agent as compared to the control group. The result also showed that the dose of 146.97 mg/kg expressed effective inhibition than the lower doses as shown on Table 5.

**Table 4:** Effect of ethanolic leaf extract of *Aspilia africana* on carrageenan induced paw edema in rats

| Groups | Treatments (mg/kg) | Initial | 0.5 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr |
|---|---|---|---|---|---|---|---|---|
| I | -ve control (saline 10 ml/kg) | 3.64 ± 0.08 | 6.75 ± 0.09 | 6.75 ± 0.08 | 6.76 ± 0.09 | 6.80 ± 0.08 | 6.83 ± 0.07 | 6.87 ± 0.08 |
| II | +ve control (ASA 100) | | 3.63 ± 0.04 | 6.20 ± 0.13 | 5.93 ± 0.11 | 5.74 ± 0.11* | 5.49 ± 0.09* | 5.24 ± 0.08* |
| III | Plant Extract (48.99) | | 3.61 ± 0.09 | 6.73 ± 0.03 | 6.66 ± 0.03 | 6.57 ± 0.04* | 6.43 ± 0.05* | 6.19 ± 0.06* |
| IV | Plant Extract (97.98) | | 3.49 ± 0.07 | 6.58 ± 0.14 | 6.22 ± 0.16 | 6.12 ± 0.15* | 6.03 ± 0.16* | 5.85 ± 0.15* |
| V | Plant Extract (146.97) | | 3.58 ± 0.10 | 6.71 ± 0.16 | 6.51 ± 0.19* | 6.00 ± 0.17* | 5.84 ± 0.14* | 5.70 ± 0.14* |

Results are expressed as mean (± SEM) of five replicates. *significant (p=0.05) when compared to control group. Sample size (n=5). ASA = Aspirin.

**Table 5:** Percentage inhibition of paw edema volume in *Aspilia Africana*

| Group | Treatment (mg/kg) | Percentage inhibition at various times intervals | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0.5 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr |
| III | Plant Extract (48.99) | 0.30 | 1.33 | 2.81 | 5.44 | 7.61 | 9.90 |
| IV | Plant Extract (97.98) | 2.52 | 7.85 | 9.47 | 11.32 | 13.47 | 14.85 |
| V | Plant Extract (146.97) | 0.59 | 3.56 | 7.84 | 11.76 | 14.49 | 17.03 |

The qualitative and quantitative phytochemical screening gave the estimation of the crude yields of chemical constituents in *Aspilia africana* revealed that it is rich in flavonoids, tannin, saponin cardiac glycoside and anthraquinone. However, alkaloids were not present in *Aspilia africana* while other constituents were present. The absence of alkaloids in *Aspilia africana* is in contradiction with the work of Abii and Onuoha [16] who expressed the prominence of this constituent in Umudike, Abia State; while Essiett and Akpan [17] reported its absence of alkaloids in *Aspilia africana* in Uyo, Akwa Ibom State in connection with this present study. This suggests that geographical location has a great influence in bioactive constituents present in plants.

The presence of phytochemicals in *A. africana* infers a possibility of medicinal efficiency of this plant. Phytochemicals are non-nutritive chemicals that contain protective, disease preventive compound. They are naturally occurring compounds in fruits, vegetables, legumes and grains. These compounds are associated with prevention and treatment of diseases such as cancer, cardiovascular diseases and hypertension [18]. The results of qualitative and quantitative analyses clearly indicated pronounce presence of tannins, alkaloids, saponins, cardiac glycosides and flavonoids. Tannins have been reported to provide protection against microbial degradation of dietary proteins [19], tannins also act as antioxidants; may inhibit enzymes that activate carcinogens [20]. Saponins have been reported to cleanse and purify blood [21], control human cardiovascular disease and reduce cholesterol levels [19]. Saponins have exhibited significant inhibition on the proliferation of cultured cell, antileishmanial, anti-inflammatory and antibacterial activity [22].The flavonoids have been reported to have antiviral, anti-allergic, anti-platelet, anti inflammatory, antitumor and antioxidant activities [23].

Iintraperitoneal administration of ethanolic leaf extracts of *Aspilia africana* showed that mortality could reach 100% when mice are exposed to 1,000 mg/kg, 2,000 mg/kg or more. Hashemi *et al.* [24] earlier reported that oral administration of 2,000 mg/kg of aqueous extract of some selected herbs was non-toxic to birds.

The search for new anti-inflammatory agents from the huge array of medicinal plant resources is on the increase. This is because medicinal plants may hold assurance for the discovery of novel therapeutic agents capable of suppressing or reducing inflammation with limited adverse effects. In the present study, the anti-inflammatory activity of *Aspilia africana* was investigated using the carrageenan. Carrageenan-induced rat paw edema is a widely used test to determine anti-inflammatory activity and constitutes a simple and routine animal model for evaluation of pain at the site of inflammation without any injury or damage to the inflamed paw [25]. It is believed to be triphasic.

The first phase (0– 2h) of the carrageenan model is mainly mediated by histamine and serotonin; biochemically, the metabolic balance was sharply disturbed in favour of catabolism, this raised the osmotic pressure, attracting extra fluid into the tissue, edema; and also free heat liberated from lytic and other exothermic reactions such as decarboxylation, desamination or glucose fermentation significantly elevate tissue temperature on its way out of the body at the second phase [26]. The second phase (2–3 h) is mediated by bradykinin, leukotrienes, polymorphonuclear cells and the last phase (3–5h) which begins after the bradykinin phase and is consecutive to the liberation of prostaglandinsproduced by tissue macrophages [27].

The result from this study indicates that ethanolic leaf extract of *Aspilia africana* showed significant inhibitory effect on rat paw edema development from the second phase to the third phase at 2-5 hours. This suggests that the extracts possibly act by the inhibition of the action of histamine and serotonin and also suggests a possible inhibition of cyclooxygenase synthesis by the extract, because the carrageenan inflammatory model basically reflects the actions of prostaglandins. This effect is similar to that produced by non steroidal anti inflammatory drugs such as aspirin, ibuprofen and indomethacin, whose mechanism of action is inhibition of the cyclooxygenase enzyme.

## Conclusion

The results from this study revealed that the leaves *Aspilia africana* contain phytochemical constituents such as saponins, tannins, combined anthraquinone, cardiac glycocides, terpenes and flavonoids in appreciable amounts; also that the leaves of *A. africana* are non -toxic at a particular dose. Results of this study revealed that the ethanolic leaf extracts of the leaves of *A. africana* obviously inhibits inflammation of hind paw

edema of albino rats induced by carrageenan. Therefore, the efficacy and efficiency of leaves of *A. africana* is recommended therapeutically, locally, traditionally and pharmaceutically for inhibition and treatment of inflammation. However, *Aspilia africana* is slightly toxic at high dose and so it should be taken at an average dose to enhance its effectiveness because of its reported effect on female reproductive system.

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