Antiinflammatory and antipyretic activities of *Clausena anisata*

Jude Efiom Okokon¹, Anwanga Effiong Udoh¹, Ukeme Essien Andrew¹, Louis Uchechukwu Amazu²

¹Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.
²Department of Pharmacology and Therapeutics, College of Medicine, Enyars Enywerem University, Orlu Campus, Nigeria.

*Corresponding author: judeefiom@yahoo.com; Tel: +234-802-3453678

Received: 5 July 2012, Revised: 16 July 2012, Accepted: 19 July 2012

**Abstract**

Anti-inflammatory and antipyretic activities of leaf extract of *Clausena anisata* were evaluated to ascertain the folkloric claim of its anti-inflammatory and antipyretic effects. The crude leaf extract (39–117mg/kg) of *C. anisata* was investigated for anti-inflammatory and antipyretic activities using various experimental models. The extract caused a significant (p<0.05–0.001) dose-dependent reduction of inflammation and fever induced by different agents used. The anti-inflammatory and antipyretic effects of this plant may in part be mediated through the chemical constituents of the plant.

**Keywords:** *Clausena anisata*, Anti-inflammatory, antipyretic

**Introduction**

*Clausena anisata* (Wild) Hook. F. ex Benth. (Rutaceae)(syn. *Clausena abyssinica* (Engl.) Engl., *Clausena inequalis* (DC.) Benth.) is a tropical shrub or tree up to 10 meters high growing in and on evergreen forests. It is commonly known as ‘mbiet ekpene’ by the Ibibios of Niger Delta region of Nigeria. The plant is traditionally use as effective remedies for worms infections, respiratory ailments, hypertension, malaria, fever, rheumatism, arthritis and other inflammatory conditions, headaches, pains, toothaches, convulsions and others (Hutchings et al.,1996). The Ibibios use the plant to treat measles (Ajibesin et al.,2005), malaria, pains and inflammations (Uwaifo, 1984; Philip Ikpe, personnal communication).The plant has been reported to contain coumarins, limonoids, carbazole alkaloids, monoterpenoids furanocoumarin lactones (Lakashmi et al.,1984) and essential oils (Cakrabirty and Chowdhury, 1995; Ngadjui et al.,1989; Ito et al., 2000; Usman et al., 2010). Reports of antimicrobial (Gundidza et al.,1994), antibacterial (Senthikumar and Venkatesalu,2009), antidiabetic (Ojewole, 2002), anticonvulsant (Makanju, 1983), antitumor promoting (Ito et al., 2000) and in vivo antiplasmodial and analgesic (Okokon et al., 2012) activities have been published. This study was carried out to evaluate the anti-inflammatory and antipyretic activities of this
plant to confirm its use traditionally to treat inflammatory diseases and fever.

**Materials and methods**

*Plant Materials*

The fresh leaves of *Clausena anisata* were collected from the Ukap in Ikono area of Akwa Ibom State and were identified and authenticated as *Clausena anisata* (Wild) Hook .F. ex Benth (Rutaceae) by Dr. (Mrs.) Margaret Bassey of the Department of Botany and Ecological studies, University of Uyo and deposited at University of Uyo herbarium (UUH 653).

*Extraction*

The leaves of the plant were air-dried, pulverized using pestle and mortar and cold-macerated for 72 hours using ethanol. The liquid ethanolic extract that was obtained by filtration was concentrated and evaporated to dryness in vacuo at 40°C using rotary evaporator. The ethanolic extract was stored at -4°C until used.

*Phytochemical Screening*

Phytochemical screening of the crude leaf extract was carried out employing standard procedures and tests (Trease and Evans, 1989, Sofowora, 1993), to reveal the presence of chemical constituents such as alkaloids, flavonoids, tannins, terpenes, saponins, anthraquinones, reducing sugars, cardiac glycosides among others.

*Animals*

The animals (Swiss albino mice) both male and female that were used for these experiments were obtained from University of Uyo animal house. The animals were housed in standard cages and were maintained on a standard pelleted Feed (Guinea Feed) and water *ad libitum*. Permission and approval for animal studies were obtained from College of Health Sciences Animal Ethics committee, University of Uyo.

*Determination of Median Lethal dose (LD₅₀)*

The median lethal dose (LD₅₀) of the extract was estimated using albino mice by intraperitoneal (i.p) route using the method of Miller and Tainter (1944). This involved intraperitoneal administration of different doses of the extract (100 – 1000 mg/kg) to groups of three mice each. The animals were observed for manifestation of physical signs of toxicity such as writhing, decreased motor activity, decreased body/limb tone, decreased respiration and death.

*Evaluation of anti-inflammatory activity of the extract*

*Carrageenin-induced mice hind paw oedema*

Increase in the mice hind paw linear circumference induced by planar injection of the phlogistic agent was used as the measure of acute inflammation (Winter *et al.*, 1962). Adult albino mice of either sex were used after 24 hours fast and deprived of water only during
experiment. Inflammation of the hind paw was induced by injection of 0.1ml of freshly prepared carrageenin suspension in normal saline into the sub planar surface of the hind paw. The linear circumference of the injected paw was measured before and 0.5, 1, 2, 3, 4 and 5 hrs after administration of phlogistic agent. For routine drug testing, the increase in paw circumference 0.5, 1, 2, 3, 4 and 5 hrs after administration of phlogistic agent was adopted as the parameter for measuring inflammation (Winter, et al., 1962; Akah and Nwambie, 1994; Ekpendu et al., 1994, Besra et al., 1996). Edema (inflammation) was assessed as difference in paw circumference between the control and 0.5, 1, 2, 3, 4 and 5 hrs after administration of phlogistic agent [Hess and Milonig, 1992]. The extract (39, 78 and 117 mg/kg i.p) was administered to various groups of mice, 1 hr before inducing inflammation. Control mice received carrageenin while reference group received ASA (100 mg/kg). The average (mean) oedema was assessed by measuring with vernier calipers.

**Egg-albumin induced inflammation**

Inflammation was induced in mice by the injection of egg albumin (0.1ml, 1% in normal saline) into the sub planar tissue of the right hind paw (Akah and Nwambie, 1994). The linear circumference of the injected paw was measured before and 0.5, 1, 2, 3, 4 and 5 hrs after the administration of the phlogistic agent. The leaf extract (39, 78 and 117 mg/kg i.p) and ASA (100 mg/kg orally) were administered to 24 hrs fasted mice 1 hr before the induction of inflammation. Control group received 10 ml/kg of distilled water orally. Edema (inflammation) was assessed as the difference in paw circumference between the control and 0.5, 1, 2, 3, 4 and 5 hrs after the administration of the phlogistic agent (Hess and Milonig, 1972). The average (mean) edema was assessed by measuring with vernier calipers.

**Xylene–induced ear oedema**

Inflammation was induced in mice by topical administration of 2 drops of xylene at the inner surface of the right ear. The xylene was left to act for 15 mins. C. anisata leaf extract (39, 78, and 117 mg/kg i.p), dexamethasone (4 mg/kg) and distilled water (0.2 ml/kg) were orally administered to various groups of mice 30 minutes before the induction of inflammation. The animals were sacrificed under light anaesthesia and the left ears cut off. The difference between the ear weights was taken as the oedema induced by the xylene (Tjolsen et al., 1992).

**Evaluation of antipyretic activity of the extract**

**2,4–Dinitrophenol (DNP) induced pyrexia**

Adult albino rats (120–165 g) of both sexes fasted for 24 hours but allowed water ad libitum were used for the experiment. They were randomized into groups of 6 rats each. DNP (10 mg/kg, i.p) was administered to the rats after obtaining the basal rectal temperatures. Hyperthermia developed within 30 min of DNP administration. Different doses of extract (39, 78, and 117 mg/kg i.p), aspirin (100 mg/kg) and distilled water (10 ml/kg, orally) were administered respectively to the treatment and control groups of animals. Rectal temperatures of the animals were obtained at an hour interval for 5 hrs (Backhouse et al., 1994; Winter et al., 1962; Mbagwu et al., 2007).
D-amphetamine induced pyrexia

Adult albino rats (120–175 g) of both sexes fasted for 24 hours but allowed water ad libitum were used for the experiment. They were randomized into groups of 6 rats each. Amphetamine (5 mg/kg, i.p) was administered to the animals after obtaining basal temperatu-res. Hyperthermia developed 0.5hrs following amphetamine administration. The extract (39, 78 and 117 mg/kg, i.p) aspirin (100 mg/kg orally) and distilled water (10 ml/kg orally) were administered to the animals at peak hyperthermia. Rectal temperatures were obtained at 1hr interval for 5hrs (Blackhouse et al., 1994; Bamgbose and Noamesi, 1981; Mbagwu et al., 2007).

Yeast-induced pyrexia

Adult albino rats (130–170 g) of both sexes fasted for 24 hours but allowed water ad libitum were used for the experiment. They were randomized into groups of 6 rats each. At zero hour, the basal temperature of the rats was taken using digital clinical thermometer. Thereafter, each animal was administered subcutaneously with 20% W/V aqueous suspension of yeast at a volume of 10 ml/kg (Gural et al., 1955, Okokon and Nwafor, 2010). At suitable intervals beginning one hour after yeast injection, rectal temperature of animals were taken, animals with increase of 1°C were selected and grouped for the study. The extract understudy was administered i.p. after the pyrogen at doses of 39, 78 and 117 mg/kg to respective groups of rats. The control group received distilled water (10 ml/kg) and the reference group administered with ASA (100 mg/kg) both intraperitoneally. The rectal temperature of the groups was taken at 1hr interval for 5hrs.

Statistical analysis and data evaluation

Data obtained from this work were analyzed statistically using Students’ t-test and ANOVA (One- or Two-way) followed by a post test (Tukey-Kramer multiple comparison test). Differences between means will be considered significant at 1 % and 5 % level of significance i.e P ≤ 0.01and 0.05.

Results

Phytochemical screening

The phytochemical screening of the ethanolic extract of the leaves of Clausena anisata revealed the presence of cardiac glycosides, tannins, saponins, terpenes and flavonoids.

Acute toxicity

The median lethal dose (LD50) was calculated to be 393.7± 25.64 mg/kg. The physical signs of toxicity included excitation, paw licking, increased respiratory rate, decreased motor activity, gasping and coma which was followed by death.
Anti-inflammatory activity

Carragenin-induced oedema in mice

The effect of ethanolic leaf extract of *C. anisata* on carragenin-induced oedema is shown in figure 1. The extract exerted a significant (P<0.05–0.001) antiinflammatory effect in a dose–dependent manner which was comparable to the standard drug, ASA,100mg/kg.

Egg albumin- induced oedema

Administration of leaf extract of *C. anisata* on egg albumin - induced oedema in mice caused a significant (p<0.05–0.001) dose-dependent anti-inflammatory effect against oedema caused by egg albumin .The effect was comparable to that of standard drug, ASA (100 mg/kg) (Figure 2).

![Figure 1: Effect of *Clausena anisata* leaf extract on carrageenin-induced oedema in mice.](image1)

![Figure 2. Effect of *Clausena anisata* leaf extract on egg-albumin induced oedema in mice.](image2)
Xylene-induced ear edema

Anti-inflammatory effect of leaf extract of *C. anisata* against xylene-induced ear oedema in mice is shown in Table 1. The extract exerted a dose-dependent anti-inflammatory effect which was significant ($P<0.001$) when compared to control. The effect was incomparable to that of the standard drug, dexamethasone (4.0 mg/kg).

Antipyretic test

Dinitrophenol induced pyrexia

The antipyretic effect of the extract on DNP induced pyrexia is shown in Table 2. Administration of the leaf extract of *C. anisata* (39, 78 and 117 mg/kg) in the presence of the pyrogen caused a significant ($P<0.05–0.001$) reduction in the temperatures of the extract treated rats when compared with the control. The antipyretic effect was dose-dependent and comparable to that of the standard drug, ASA (100 mg/kg).

Amphetamine–induced pyrexia

The effect of the extract on amphetamine induced pyrexia is shown in Table 3. The extract exerted a significant ($P<0.05–0.001$) dose-dependent antipyretic effect when compared to control. The antipyretic effect of the extract was comparable to that of the standard, ASA (100 mg/kg).

Yeast-induced pyrexia

Table 4 shows the effect of the extract against yeast-induced pyrexia. There was a dose-dependent reduction in the temperature of rats treated with the leaf extract. The reductions

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Weight of right ear (g)</th>
<th>Weight of right ear (g)</th>
<th>Increase in ear weight (g)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.075 ± 0.01</td>
<td>0.043 ± 0.00</td>
<td>0.032 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Extract 450</td>
<td>0.050 ± 0.01</td>
<td>0.041 ± 0.01</td>
<td>0.009 ± 0.01$^a$</td>
<td>71.87</td>
</tr>
<tr>
<td>Extract 450</td>
<td>0.048 ± 0.01</td>
<td>0.042 ± 0.01</td>
<td>0.006 ± 0.01$^a$</td>
<td>81.25</td>
</tr>
<tr>
<td>Extract 450</td>
<td>0.043 ± 0.01</td>
<td>0.038 ± 0.01</td>
<td>0.005 ± 0.00$^a$</td>
<td>84.37</td>
</tr>
<tr>
<td>Asa 100</td>
<td>0.040 ± 0.01</td>
<td>0.036 ± 0.01</td>
<td>0.004 ± 0.00$^a$</td>
<td>87.50</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. Significant at $^aP < 0.05$, $^bP < 0.001$ when compared to control. n = 6.
Table 3. Effect of Heinsia crinata leaf extract on amphetamine induced pyrexia in mice.

<table>
<thead>
<tr>
<th>Time interval (hr)</th>
<th>Dose (mg/kg)</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>34.50 ±</td>
<td>36.30 ±</td>
<td>36.82 ±</td>
<td>36.80 ±</td>
<td>36.76 ±</td>
<td>36.71 ±</td>
<td>36.30 ±</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.11</td>
<td>0.10</td>
<td>0.13</td>
<td>0.11</td>
<td>0.10</td>
<td>0.13</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extract 450</td>
<td>34.45 ±</td>
<td>36.45 ±</td>
<td>36.30 ±</td>
<td>35.86 ±</td>
<td>35.45 ±</td>
<td>35.28 ±</td>
<td>34.63 ±</td>
</tr>
<tr>
<td></td>
<td>0.16</td>
<td>0.10</td>
<td>0.15</td>
<td>0.15</td>
<td>0.14a</td>
<td>0.14a</td>
<td>0.17a</td>
<td>0.17a</td>
</tr>
<tr>
<td></td>
<td>Extract 450</td>
<td>34.68 ±</td>
<td>36.58 ±</td>
<td>35.98 ±</td>
<td>35.75 ±</td>
<td>35.25 ±</td>
<td>34.55 ±</td>
<td>34.46 ±</td>
</tr>
<tr>
<td></td>
<td>0.23</td>
<td>0.18</td>
<td>0.22a</td>
<td>0.22a</td>
<td>0.30a</td>
<td>0.30a</td>
<td>0.31a</td>
<td>0.29a</td>
</tr>
<tr>
<td></td>
<td>Extract 450</td>
<td>34.38 ±</td>
<td>36.68 ±</td>
<td>35.87 ±</td>
<td>35.53 ±</td>
<td>35.18 ±</td>
<td>34.46 ±</td>
<td>34.40 ±</td>
</tr>
<tr>
<td></td>
<td>0.22</td>
<td>0.29</td>
<td>0.16</td>
<td>0.21a</td>
<td>0.20a</td>
<td>0.17a</td>
<td>0.13a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Asa 100</td>
<td>34.55 ±</td>
<td>36.76 ±</td>
<td>36.50 ±</td>
<td>36.26 ±</td>
<td>35.76 ±</td>
<td>35.23 ±</td>
<td>35.06 ±</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0.17</td>
<td>0.12a</td>
<td>0.19a</td>
<td>0.20a</td>
<td>0.21a</td>
<td>0.22a</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. Significant at aP < 0.05, b P < 0.001 when compared to control. n = 6.

Table 4. Effect of Heinsia crinata leaf extract on yeast induced pyrexia in mice.

<table>
<thead>
<tr>
<th>Time interval (hr)</th>
<th>Dose (mg/kg)</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>35.01 ±</td>
<td>36.93 ±</td>
<td>36.93 ±</td>
<td>36.86 ±</td>
<td>36.75 ±</td>
<td>36.58 ±</td>
<td>36.55 ±</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.11</td>
<td>0.15</td>
<td>0.11</td>
<td>0.08</td>
<td>0.07</td>
<td>0.06</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extract 450</td>
<td>34.73 ±</td>
<td>36.91 ±</td>
<td>36.87 ±</td>
<td>36.51 ±</td>
<td>36.01 ±</td>
<td>35.61 ±</td>
<td>35.21 ±</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td>0.14</td>
<td>0.11</td>
<td>0.20</td>
<td>0.21b</td>
<td>0.17a</td>
<td>0.17b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extract 450</td>
<td>34.78 ±</td>
<td>36.85 ±</td>
<td>36.76 ±</td>
<td>36.50 ±</td>
<td>36.10 ±</td>
<td>35.55 ±</td>
<td>35.33 ±</td>
</tr>
<tr>
<td></td>
<td>0.12</td>
<td>0.14</td>
<td>0.12</td>
<td>0.09a</td>
<td>0.04b</td>
<td>0.06a</td>
<td>0.05a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extract 450</td>
<td>34.83 ±</td>
<td>36.85 ±</td>
<td>36.65 ±</td>
<td>36.23 ±</td>
<td>35.76 ±</td>
<td>35.43 ±</td>
<td>35.18 ±</td>
</tr>
<tr>
<td></td>
<td>0.09</td>
<td>0.08</td>
<td>0.08</td>
<td>0.05a</td>
<td>0.03b</td>
<td>0.08a</td>
<td>0.06a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Asa 100</td>
<td>35.03 ±</td>
<td>36.05 ±</td>
<td>36.56 ±</td>
<td>36.10 ±</td>
<td>35.66 ±</td>
<td>35.46 ±</td>
<td>35.24 ±</td>
</tr>
<tr>
<td></td>
<td>0.08</td>
<td>0.11</td>
<td>0.05</td>
<td>0.12b</td>
<td>0.05b</td>
<td>0.06a</td>
<td>0.06a</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. Significant at aP < 0.05, b P < 0.001 when compared to control. n = 6.

causd by the extract was significant (P<0.005 – 0.001) when compared to control and comparable to that of the standard drug, ASA (100 mg/kg).

Discussion

*Clausena anisata* is use traditionally by the Ibibios of Niger Delta regions of Nigeria in the treatment of inflammatory conditions like pains, fever, arthritis and haemorrhoids (Hutchings et al., 1996). The present study was carried out to evaluate these properties scientifically using different experimental models.

In the carragenin induced oedema, the extract (39 – 117 mg/kg) exerted pronounced effect at the early stage of inflammation (1-2hr) indicating effect probably on histamine, serotonin and kinins that are involved in the early stage of carragenin induced oedema (Vane and Booting,1987). The extract also reduced later stage of the oedema maybe due to its ability to inhibit prostaglandin which is known to mediate the second phase of carragenin induced inflammation (Vane and Booting,1987). However, ASA (100 mg/kg) a prototype NSAID, a cyclooxygenase inhibitor whose mechanism of action involves inhibition of prost-aglandin, inhibited significantly the paw swelling due to carragenin injection.

The extract also inhibited egg albumin-induced oedema demonstrating that it can inhibit inflammation by blocking the release of histamine and 5-HT, two mediators that are
released by egg albumin (Nwafor et al., 2007). However, ASA, a cyclooxygenase inhibitor reduced significantly oedema produced by egg albumin.

The leaf extract exerted considerable inhibition of ear oedema caused by xylene in a dose-dependent manner. This suggest the inhibition of phospholipase A2 which is involve in the pathophysiology of inflammation due to xylene (Lin et al., 1992). However, dexamethasone, a steroidal antiinflammatory agent also produced significant reduction in the mean right ear weight of positive control rats indicating an inhibition of PLA2. Flavonoids are reported to be involved in antiinflammatory activity of plants (Parmer and Gosh, 1978). These have been found to be present in the extract.

In this study, the extract was observed to inhibit greatly DNP-, amphetamine and yeast-induced pyrexia. The extract is likely to reduce pyrexia by reducing brain concentration of prostaglandin E2 especially in the hypothalamus through its action on COX-3 (Botting and Ayoub, 2005) or by enhancement of the production of the body’s own antipyretic substances like vasopressin and arginine (Chandrasekharan, 2002).

In conclusion, the results of this study support the ethnobotanical use of the plant in the treatment of febrile illnesses and inflammatory conditions. Further investigation is being advocated especially in elucidating cellular mechanisms and establishing structural components of the active ingredients with a view of standardizing them.

Conflict of interest

There is no conflict of interest associated with the authors of this paper.

Acknowledgement

The authors are grateful to Mr Enefiok Ukpong of Pharmacology and Toxicology Department for his technical assistance.

References


Okokon et al.


