



Anti-inflammatory activity of leaf extract of *Solanum anomalum*

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ABSTRACT

Background & Aim: *Solanum anomalum* Thonn. ex Schumach. (family *Solanaceae*) is a shrub growing up to 2 metres tall. The edible fruits are gathered from the wild and consumed locally and the leaves are used locally in the treatment of various diseases. Evaluation of anti-inflammatory activity of leaf extract of *Solanum anomalum* was carried out to ascertain its uses in traditional medicine.

Experimental: The crude leaf extract (70 – 210 mg/kg) of *Solanum anomalum* was investigated for anti-inflammatory activity using various experimental models; carragenin, egg albumin and xylene induced oedema.

Results: The extract (70 -210 mg/kg) caused a significant ($p < 0.05 - 0.001$) dose-dependent reduction of inflammations caused by carragennin, egg albumin and xylene. The anti-inflammatory effect of this plant may in part be mediated through the chemical constituents of the plant.

Recommended applications/industries: The plant, *Breynia nivosa*, possesses antiinflammatory property which can be exploited in the treatment of inflammatory diseases.

1. Introduction

Solanum anomalum Thonn. ex Schumach. (family *Solanaceae*) is a shrub growing up to 2 metres tall. The stem, branches and midribs of the leaves are usually armed with prickles up to 5 mm long. The edible fruits are gathered from the wild and consumed locally. Both the fruits and the leaves are used medicinally. The plant is sometimes cultivated or semi-cultivated for its fruits. It is found in West tropical Africa - Sierra Leone to southern Nigeria, Cameroon and DR Congo. It is Known as 'childrens' tomatoes', they are more commonly used as a condiment in soups and sauces and the fruits are eaten raw or cooked (Burkill,

2000). The sap from the leaves and fruits is drunk, or taken by enema 1 - 2 times daily, as a treatment for leprosy and gonorrhoea (Burkill, 2000). The fruits are used as a laxative and digestive (Burkill, 2000). They are also served ground up in soups and sauces as an appetizer for sick persons, sometimes mixed with fruits of Parkia (Burkill, 2000). The crushed fruits are applied to maturate inflammations on fingers or toes (Burkill, 2000) The fruit juice is applied to sores on the ears to alleviate pain (Bukanya and Hall, 1988). Ofor and Ubengama (2015) reported the antidiabetic activity of the fruit of this plant. Although there is a little information on the fruit of this plant, there is no report of phytochemical constituents and biological activity of the leaf of *S. anomalum*. We report in this study the

phytochemical composition and antiinflammatory activity of the leaf extract of the plant.

2. Materials and Methods

2.1. Plants collection

The plant material *Solanum anomalum* (leaves) were collected in compounds in Uruan area, Akwa Ibom State, Nigeria in August, 2015. The plant was identified and authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Herbarium specimen was deposited at Department of Pharmacognosy and Natural Medicine Herbarium.

2.2. Extraction

The plant parts (leaves) were washed and shade-dried for two weeks. The dried plants' materials were reduced to powder using mortar and pestle. The powdered material was soaked in 50% ethanol. The liquid filtrate was concentrated and evaporated to dryness in vacuo 40°C using rotary evaporator and stored in a refrigerator at -4°C.

2.3. Phytochemical Screening

Phytochemical screening of the crude 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.

2.4. Animals

Albino Swiss mice (19 – 28g) of either sex were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics committee, University of Uyo.

2.5. 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 Lorke (1983). 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. The number of deaths in each group within 24 hours was recorded. The LD₅₀ was calculated as geometrical means of the maximum dose producing 0% (a) and the minimum dose producing 100% mortality (b).

$$LD_{50} = \sqrt{ab}$$

2.6. Evaluation of antiinflammatory activity of the extract

2.6.1. Carrageenin – induced mice hind paw oedema.

Adult albino male mice were used after 24 hours fast and deprived of water only during experiment. Inflammation of the hind paw was induced by injection of 0.1 ml 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. The increase in paw circumference post 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; Nwafor *et al.*, 2010). The difference in paw circumference between the control and 0.5, 1, 2, 3, 4 and 5 hrs after administration of phlogistic agent was used to assess inflammation (Hess and Milonig, 1992). The extract (70, 140 and 210 mg/kg i.p) was administered to various groups of 6 mice each, 1h 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. Average inflammation/oedema (C_t – C₀) was calculated for each dose (Oriowo, 1982; Akah and Njike, 1990).

2.6.2. 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; Okokon and Nwafor, 2010). 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 (70, 140 and 210 mg/kg i.p) and ASA (100 mg/kg orally) were administered to groups (n=6) of 24 h fasted mice 1 h 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 post administration of the phlogistic agent (Hess and Milonig, 1972). The average (mean) edema was assessed by measuring with vernier calipers. Average inflammation/oedema ($C_t - C_0$) was calculated for each dose (Oriowo, 1982; Akah and Njike, 1990).

2.6.3. 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. *Solanum anomalum* leaf extract (70, 140 and 210 mg/kg i.p), dexamethasone (4 mg/kg) and distilled water (0.2 ml/kg) were orally administered to various groups (n=6) of mice 1 h 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; Okokon and Nwafor, 2010).

2.7. Statistical analysis and data evaluation

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

3. Results and discussion

3.1. Phytochemical screening

The phytochemical screening of the ethanol extract of the leaf of *Solanum anomalum* revealed the presence of alkaloids, cardiac glycosides, tannins, saponins, terpenes and flavonoids .

3.2. Determination of Median lethal dose (LD_{50})

The median lethal dose (LD_{50}) was calculated to be 724.56 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.

3.3. Carragenin-induced oedema in mice

The effect of ethanol leaf extract of *Solanum anomalum* on carragenin-induced oedema is as shown in Table 1. The extract (70 - 210 mg/kg) exerted a significant ($P < 0.05 - 0.001$) anti-inflammatory effect in

a dose –dependent manner. The activity of the highest dose was more than that of the standard drug, ASA, 100 mg/kg (Table 1a and 1b).

3.4. Egg albumin- induced oedema

Administration of extract of *Solanum anomalum* (70 - 210 mg/kg) 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 incomparable to that of standard drug, ASA (100 mg/kg) (Table 2a and 2b).

3.5. Xylene- induced ear edema

Anti-inflammatory effect of crude extract of *Solanum anomalum* against xylene-induced ear oedema in mice is shown in Table 3. The extract exerted a dose-dependent anti-inflammatory effects that were significant ($P < 0.05 - 0.01$) but incomparable to that of the standard drug, dexamethasone (4.0 mg/kg).

In this study, the ethanol leaf extract of *Solanum anomalum* was evaluated for anti-inflammatory activity using various experimental models.

In the carragenin-induced oedema, the extract (70 - 210 mg/kg) was observed to have exerted significant effect at the early stage of inflammation (1-2 hr) indicating effect probably on histamine, serotonin and kinnins that are involved in the early stage of carragenin-induced oedema (Vane and Booting, 1987). The extract further reduction of the 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 prostaglandin, produced a considerable inhibition of the paw swelling induced by 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 extract exerted a significant ($P < 0.01$) inhibition of ear oedema caused by xylene only at all doses of the extract, suggesting the inhibition of phospholipase A_2 which is involve in the pathophysiology of inflammation due to xylene (Lin *et al.*, 1992).

Table 1a. Effect of *Solanum anomalum* leaf extract on carrageenin- induced oedema in rats.

Treatment/ Dose (mg/kg)	Time intervals (hr)						
	0	0.5	1	2	3	4	5
Control	2.49 ± 0.01	3.94 ± 0.22	4.17 ± 0.23	4.00 ± 0.12	3.87 ± 0.03	3.87 ± 0.19	3.82 ± 0.02
Extract 70	2.27 ± 0.03	3.45 ± 0.28	3.28 ± 0.61	3.29 ± 0.25	3.06 ± 0.31	2.81 ± 0.30	2.80 ± 0.33
140	2.24 ± 0.04	3.51 ± 0.22	3.34 ± 0.16	3.06 ± 0.05	3.01 ± 0.15	2.74 ± 0.13	2.66 ± 0.14
210	2.32 ± 0.03	3.57 ± 0.26	3.27 ± 0.14	3.22 ± 0.08	3.13 ± 0.15	2.79 ± 0.21	2.62 ± 0.16
ASA 100	2.41 ± 0.06	2.89 ± 0.04	2.59 ± 0.05 ^b	2.75 ± 0.02 ^a	2.60 ± 0.02 ^a	2.57 ± 0.08	2.51 ± 0.09

Data are expressed as mean ± SEM. Significant at ^ap<0.05; ^bp< 0.01 when compared to control. n = 6.

Table 1b. Effect of *Solanum anomalum* leaf extract on carrageenin induced oedema in rats.

Treatment/ Dose (mg/kg)	Average inflammation/oedema (mm) ± SEM					
	0.5hr	1hr	2hr	3hr	4hr	5hr
Control	1.45 ± 0.02	1.68 ± 0.04	1.51 ± 0.03	1.30 ± 0.11	1.25 ± 0.17	1.33 ± 0.02
Extract 70	1.18 ± 0.18	1.04 ± 0.10	1.02 ± 0.12	0.79 ± 0.21	0.54 ± 0.18 ^a	0.53 ± 0.21
140	1.27 ± 0.05	1.10 ± 0.06	1.03 ± 0.07	0.77 ± 0.15 ^a	0.50 ± 0.09 ^a	0.42 ± 0.03 ^c
210	1.25 ± 0.02	0.95 ± 0.05	0.90 ± 0.04	0.81 ± 0.06 ^a	0.47 ± 0.06 ^a	0.30 ± 0.03 ^c
ASA 100	0.68 ± 0.01	0.38 ± 0.01 ^c	0.54 ± 0.01 ^c	0.39 ± 0.01 ^c	0.36 ± 0.01 ^c	0.30 ± 0.01 ^c

Data are expressed as mean ± SEM. Significant at ^cP < 0.001 when compared to control. n = 6.

Table 2a. Effect of *Solanum anomalum* leaf extract on egg- albumin induced oedema in mice.

Treatment/ Dose (mg/kg)	Time intervals (hr)						
	0	0.5	1	2	3	4	5
Control	2.65 ± 0.07	3.55 ± 0.25	3.50 ± 0.06	3.45 ± 0.11	3.41 ± 0.07	3.35 ± 0.07	3.25 ± 0.06
Extract 70	2.38 ± 0.03	3.24 ± 0.53	3.06 ± 0.29	2.82 ± 0.30	2.70 ± 0.25	2.59 ± 0.33	2.56 ± 0.41
140	2.34 ± 0.08	3.44 ± 0.12	3.30 ± 0.20	3.16 ± 0.20	2.93 ± 0.27	2.71 ± 0.19	2.58 ± 0.24
210	2.33 ± 0.01	3.37 ± 0.12	3.09 ± 0.11	2.87 ± 0.11	2.80 ± 0.18	2.67 ± 0.13	2.57 ± 0.10
ASA 100	2.61 ± 0.06	2.99 ± 0.16 ^a	2.89 ± 0.02 ^a	2.84 ± 0.02 ^c	2.76 ± 0.03 ^c	2.64 ± 0.05 ^b	2.64 ± 0.05 ^c

Data are expressed as mean ± SEM. Significant at ^ap<0.05; ^bp< 0.01, ^cp< 0.001 when compared to control. n = 6.

Table 2b. Effect of *Solanum anomalum* leaf extract on egg-albumin induced oedema in rats.

Treatment/ Dose (mg/kg)	Average inflammation/oedema (mm) ± sem					
	0.5hr	1hr	2hr	3hr	4hr	5hr
Control	1.68 ± 0.05	1.68 ± 0.04	1.52 ± 0.04	1.30 ± 0.11	1.25 ± 0.17	1.33 ± 0.02
Extract						
70	0.86 ± 0.21	0.68 ± 0.15	0.44 ± 0.15	0.32 ± 0.12	0.21 ± 0.04 ^a	0.18 ± 0.03
140	1.10 ± 0.12	0.96 ± 0.05	0.78 ± 0.05	0.59 ± 0.10 ^a	0.37 ± 0.05 ^a	0.24 ± 0.11 ^c
210	1.04 ± 0.03	0.76 ± 0.07	0.54 ± 0.03	0.47 ± 0.02 ^a	0.34 ± 0.06 ^a	0.24 ± 0.01 ^c
ASA 100	0.83 ± 0.05	0.62 ± 0.07	0.50 ± 0.04 ^a	0.40 ± 0.01 ^a	0.24 ± 0.07 ^b	0.20 ± 0.29 ^c

Data are expressed as mean ± SEM. Significant at ^cP < 0.001 when compared to control. n = 6.

Table 3. Effect of *Solanum anomalum* leaf extract on xylene-induced ear oedema in mice.

Treatment/ Dose (mg/kg)	weight of right ear (g)	weight of left ear (g)	increase in ear weight (g)	% inhibition
Control (normal saline) 0.2ml	0.032 ± 0.002	0.090 ± 0.002	(181.25) 0.058 ± 0.002	
Extract 70	0.044 ± 0.002	0.071 ± 0.00	(61.36) 0.027 ± 0.002 ^a	53.44
140	0.042 ± 0.003	0.065 ± 0.004	(71.42) 0.030 ± 0.006 ^b	48.27
210	0.035 ± 0.003	0.063 ± 0.005	(80.00) 0.028 ± 0.002 ^c	51.72
Dexamethasone 4.0	0.036 ± 0.003	0.054 ± 0.003	(24.52) 0.018 ± 0.003 ^c	68.96

Figures in parenthesis indicate % increase in ear weight. *significant at $p < 0.05$, $p < 0.01$, $p < 0.001$ when compared with control. $n = 6$.

However, dexamethasone, a steroid anti-inflammatory agent produced significant reduction in the mean right ear weight of positive control rats indicating an inhibition of PLA₂.

The phytochemical screening shows that the leaf extract contains alkaloids, cardiac glycosides, tannins, saponins, terpenes and flavonoids.

Flavonoids are known anti-inflammatory compounds acting through inhibition of the cyclooxygenase pathway (Liang *et al.*, 1999). Some flavonoids are reported to block both the cyclooxygenase and lipoxygenase pathways of the arachidonate cascade at relatively high concentrations, while at lower concentrations they only block lipoxygenase pathway (Carlo *et al.*, 1999). Some flavonoids exert their antinociception via opioid receptor activation activity (Suh *et al.*, 1996; Rajendran *et al.*, 2000; Otuki *et al.*, 2005). Flavonoids also exhibit inhibitory effects against phospholipase A2 and phospholipase C (Middleton *et al.*, 2000), and cyclooxygenase and/or lipoxygenase pathways (Robak *et al.*, 1998).

Triterpenes have been implicated in anti-inflammatory activity of plants (Huss *et al.*, 2002; Suh *et al.*, 1998) and reports on their analgesic activities have also been published (Liu, 1995; Krogh *et al.*, 1999; Tapondjou *et al.*, 2003; Maia *et al.*, 2006). Ursolic acid is a selective inhibitor of cyclooxygenase-2 (Ringbom *et al.*, 1998). Oleanolic acid is known to exert its analgesic action through an opioid mechanism, and possibly, a modulatory influence on vanilloid receptors (Maia *et al.*, 2006).

In conclusion, the results of this study demonstrated that *Solanum anomalum* leaf possess anti-inflammatory

property and this confirms its usage in traditional medicine to treat inflammatory diseases.

4. Conclusion

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