Phytochemical Analysis and Anti-inflammatory Property of methanol extract of the leaves of *Solanum nigrum* Linn (Solanacecae)

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ABSTRACT
Solanum nigrum leaves have been claimed to be use ethnomedicinally as an anti-inflammatory agent. It is used as poultice for rheumatic and gouty pain. It is also used in cutaneous or skin inflammation and bronchitis. This present study is to analyse the phytochemical constituents of the leaves of *S. nigrum* and its anti-inflammatory activity. Phytochemical screening on the leaves indicated the presence of Tannins, Saponins, Alkaloids, reducing sugars and carbohydrates. The anti-inflammatory activity of the methanolic extract of *Solanum nigrum* was investigated using carrageneman-induced rat paw oedema in rats at doses of 100mg/kg, 200mg/kg and 400mg/kg. Results show a dose-dependent inflammatory inhibition ($p < 0.05$) between the third and fifth hours. On the basis of this study, it may be inferred that the leaves of *Solanum nigrum* has a significant ($p < 0.05$) anti-inflammatory properties and hence justifies, its ethnomedical use as an anti-inflammatory agent.

Keywords: *Solanum nigrum,* Phytochemical screening, anti-inflammatory activity, carragennan, paw oedema.

INTRODUCTION:
It is common knowledge that people, all over the world have used herbs to cure and control diseases. The treatment of rheumatic and other inflammatory conditions is an area where Nigerian traditional medicine practitioners enjoy patronage and success (Akah and Nwambie, 1994). There are many plant species used for treatment of inflammation and other disorders but information on these plants in some cases is limited (Akah and Njike, 1990).

Solanum nigrum leaves are used in many areas of Edo State as vegetables and medicinally for the various ailments such as convulsions, especially in children, stomach pain, rheumatic pain and in some areas threatened abortion. Whole plant investigation of Solanum nigrum indicates that it contains substances such as Alkaloids (Tang *et al.,* 2006), steroidal Alkaloids and steroidal saponins (Ji *et al.,* 2008, Zhou *et al.,* 2006) and glycoprotein (Heo *et al.,* 2004).

The fruits of *S. nigrum* have been shown to posess anti-tumour activity (Arthar, 1995), neuropharmacological properties (Chou, 2008), anti-oxidant properties (Lim, 2004), anti-proliferative, apoptotic and cytotoxic effects (Lee, 2003). Larvicidal, anti-inflammatory and anti-convulsant activity (Singh *et al.,* 2001) and Anti-ulcer activity (Jainu and Devi, 2006).

In this present study, phytochemical screening of the leaves was carried out and anti-inflammatory activity of the methanolic Extract of the dry leaves was investigated at different doses using carrageenan-induced paw oedema (an acute inflammatory model) in rats (Adeyemi *et al.,* 2005)

MATERIALS AND METHODS
Plant Materials
The fresh leaves of *Solanum nigrum* were collected from Iraokhor village, Etsako Central Local Government Area of Edo State, Nigeria in September, 2010 and was identified and authenticated by Professor MacDonald Idu of the Department of Botany, Faculty of Life Sciences, University of Benin, Benin city, Nigeria. The leaves were air dried for

three weeks and grounded into a powder using a mechanical grinder.

Extraction:

850g of the powdered leave sample of *S. nigrum* was macerated in methanol (2.5 L) for 48 hours with intermittent agitation at room temperature. This was then filtered using a sieve and finally through a filter paper. The filtrate was evaporated using rotary evaporator and the residue weighed (yield 3.3% w/w)

Chromatographic Analysis

Analytical thin layer chromatography (TLC, silica gel 60 F254 plates, Merck, Darmstadt,Germany, visualization: UV 254 nm) was used to monitor the best solvent system that will separate the components of the leaves of *Solanum nigrum.*

Phytochemical Test

Phytochemical screening was done for alkaloids tannins, saponins, carbohydrates, anthraquinones, flavonoids cyanogentic glycosides using standard procedure (Evans 2002; Trease and Evans, 2003)

Pharmacologic Activity

Albino rats (180-220g) of both sexes (excluding pregnant females) obtained from the animal home of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria were used. The animals were maintained under standard conditions and allowed free access to standard diet (Bendel Feed and Flower Mill-Grower Marsh) and water ad labium. The animals were allowed to acclamatise for 2 weeks and fasted over night prior to experiment. Approval for the use of the animals for anti-inflammatory experiment was obtained from the Animal ethnical committee of the Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

In the experiment, the rats were grouped (5 animals per group). Oedema was induced in the right hind paw of the rats by injection of 0.1ml of freshly prepared 1% carrageenan suspension in 0.9% saline into the subplanter tissue (Winter *et al.,* 1963).

The extract at 100-400 mg/kg or Ibuprofen 20 mg/kg was administered P.O. suspended in 10% Tween 20 by the use of an Oro -gastric tube to the rats. One hour after inducing inflammation with carrageenan, control animals received 0.2 ml of 10% Tween 20 orally (P.O.).

The paw diameter was taken, using a vernier caliper (Okunrobo et al., 2009), the measurements were made immediately before injection of carrageenan and thereafter at hourly intervals for 6 hours. The paw swelling at each time was calculated as difference between the paw diameter at time, t (D_t) and that at zero time (D_0). An average of three readings was taken. The differences between the paw diameters D_t and D_0 were recorded and analysed, the percentage inhibition was calculated for every hour as

$$\frac{C - T}{C} \times 100\%$$

Where C = mean paw diameter for control group
T = mean paw diameter for treatment group

Statistical analysis

All data were expressed as mean $\pm$ SEM and where applicable were analyzed for statistical significance using one way ANOVA followed by Dunnet's test. A *P*- value < 0.05 was considered significant.

RESULTS AND DISCUSSION

The low yield of the crude methanol extract obtained (3.3 %) is an indication that maceration is not a suitable method of extraction. Rather, soxhlet extraction which uses less quantity of extracting solvent gives a higher yield of extract and is a preferable method of extraction although there is the risk of heat destroying the thermolabile constituents in the plant, for that reason use of heat was reserved until absolutely necessary. Three distinct spots were seen on the chromatography under the UV lamp at wave length 254 nm and Rf values obtained were 0.63, 0.7, and 0.75 respectively, and the best solvent system used was methanol:chloroform (4:1). The results of the phytochemical screening carried out on the powdered leaves of *Solanum nigrum* revealed the presence of alkaloids, tannins, saponins, carbohydrates and reducing sugars (Table 1). Isoquinoline alkaloids have been shown to inhibit acetylcholinesterase and

have proved useful in the treatment of Alzheimer's disease (Pagliosa et al., 2010). They also inhibit inflammation and possess dose-dependent anti-nociceptive ability (Kupeli et al., 2002) and have been shown to inhibit the movement of second-stage larvae of *Toxocara canis* (Satou et al., 2002).

Table 1:
Phytochemical Constituents of methanolic extract of leaves of *Solanum nigrum*

Secondary metabolites	Result
Alkaloids	+
Tannins	+
Saponins	+
Anthraquinones	-
Cynogentic glycosides	-
Favonoids	-
Carbohydrates	+
Reducing sugars	+

+ = present; - = absent.

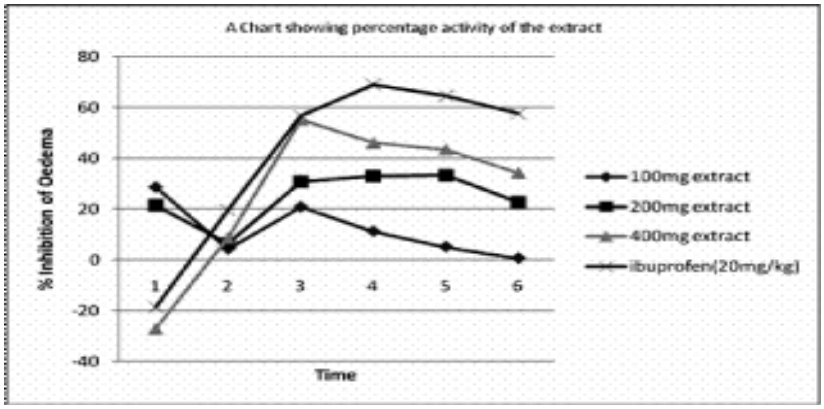
Table 2:
Effects of methanol extract of S. nigrum on carrageenan-induced rat paw oedema

Treatment	1hr	2hr	3hr	4hr	5hr	6hr
M E 100mg/kg P.O.	1.06 ± 0.01*	1.76 ± 0.07	1.90 ± 0.10	1.83 ± 0.12	1.68 ± 0.14	1.53 ± 0.12
ME 200mg/kg P.O.	1.10 ± 0.04*	1.72 ± 0.18	1.66 ± 0.10	1.38 ± 0.07	1.18 ± 0.05	1.19 ± 0.04
ME 400mg/kg	1.78 ± 0.13	1.68 ± 16	1.26 ± 0.09	1.11 ± 0.11	1.00 ± 0.11	1.00 ± 0.10
IBUPROFEN 200mg/kg P.O.	1.66 ± 0.09	1.54 ± 0.11	1.04 ± 0.07	0.64 ± 0.02	0.63 ± 0.03	0.65 ± 0.03
CONTROL (0.2ml 10% tween 20)	1.40 ± 0.03	1.84 ± 0.08	2.40 ± 0.05	2.06 ± 0.09	1.77 ± 0.12	1.54 ± 0.14

Value are expressed as Mean ± SEM $P< 0.05$ when compared with control group (n=5), ME = Methanol exract

The results revealed a relatively low yield of 3.3% w/v of the methanolic extract of *S. nigrum*. The chromatogram developed in a methanol; chloroform (4:1) system gave three (3)

Figure 1 : The Percentage Inhibitions of S. nigrum



Anti-inflammatory activity of the extract showed a dose-dependent increased in activity between 100 mg/kg – 400 mg/kg with the greatest activity of 45% inhibition obtained at 3 hours at a dose of 400mg/kg of the extract. It was also observed that the activity of the extract was time dependent with peak activity recorded for all doses between the 3rd and 4th hours. The activity of the extract increased from the first hour, peaked between the 3rd and 4th hours and thereafter progressive decrease until the 6th hour. The same pattern of activity was observed for the reference drug (Ibuprofen 20mg/kg P.O.).

Inflammation is a response of living tissue to injury which involves a complex mechanism of enzymes and mediators (Katzung, 2004). Acute inflammatory process involves 3 stages. Firstly injury causes the release of histamine and serotonin (5HT) and lasts for between one and two hours post injury. The second phase involves the release of Kinnins and lasts from the second hour to the third hour. The third phase involves the release of prostaglandins and lasts from the third to fifth hours post injury (Surrender and Mafumdar, 1995), this third phase also corresponds with the time of maximum inflammation (Duffy *et al.,* 2001)

From the statements above and assessment of the results of the anti-inflammatory potency; the extract with greater activity within the 3rd and 5th hours could be said to have had a greater inhibition on the prostaglandins phase of inflammation and to a lesser extent the initial early stages of inflammation (i.e histamine, 5HT and Kinnins phases). The reference drug (Ibuprofen) showed this same pattern of activity. Ibruprofen is a well known non steroidal anti-inflammatory drug (NSAID) it owes its anti-inflammatory activity to inhibition of prostangaldnin biosynthesis via non-selective inhibition of cycloxyegnase (CoX) enzyme. From the foregoing, it could be inferred that the extract owes its activity to inhibition of prostaglandin inhibition (the mechanism will be subjected to further investigation).

On the basis of this study, it is concluded that the methanolic extract of *S. nigrum* possess anti inflammatory action and hence justifies its ethnomedicinal use in this respect.

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