

Full Length Research Paper

# Investigation of phytochemical, anti inflammatory and anti nociceptive properties of *Ipomoea asarifolia* leaves

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*Ipomoea asarifolia* has been used in traditional medicine for treating various ailments including dysmenorrhea, without scientific verification of its effects. Phytochemical studies were carried out on samples of *I. asarifolia* to determine its pharmacognostic profile. Acetic acid-induced writhing in mice, formalin-induced pain and egg-albumin induced inflammatory tests in rats were employed to investigate anti-nociceptive and anti inflammatory properties of 70% methanolic extract of leaves. Alkaloids, saponin and phenols were present. The extract doses of 100 – 400 mg/kg i.p. significantly ( $P < 0.05$ ) reduced inflammation and pain at the late phase of the process. The derived LD<sub>50</sub> was 1,732.1 mg/kg i.p. These results justify the ethnomedicinal uses of the plant for pain relief and anti inflammation.

**Key words:** *Ipomoea asarifolia*, Anti-nociception, Anti-inflammation, Phytochemistry.

## INTRODUCTION

*Ipomoea asarifolia* (Desr.) Roem. and Schult. (Convolvulaceae) is a glabrous succulent, perennial plant trailing on the ground. It reproduces from seeds and stem shoots. It is a perennial, creeping or trailing growing on sandy soil or waste lands. It is native to tropical America but now pantropical. It is found throughout West Africa from Cameroun to Senegal, Mali and the Cape Verde Island, and Tropical Asia. It is a common weed of hydromorphic soils, low lying and inland valleys, streams and riverbanks. In Nigeria, the traditional names are 'Duman-kaada,' 'Duman-kaafi' and 'Dumang kadu' in Hausa. 'Gboro-ayaba', 'Ododo oko' and 'Ododo amu' in Yoruba.

In Senegal, it is used traditionally for various gynecological purposes such as, hemorrhage. It is also used as an ecboic and abortifacient and in general for wound dressing. It is also used in treating ophthalmias, neuralgias, headaches, and arthritic and stomach pains. In northern Nigeria, a leaf poultice is applied to guinea worm sores while the face is steamed over a hot decoction of

the plant along with husks of bulrush millet. A leaf decoction is usually taken internally as a wash for feverish chills and rheumatic pains (Burkill, 1985). The leaves are used to treat dysmenorrheal in the Middle Belt region of Nigeria (Personal Communication). The flowers of the plant contain two triacylated and tetraglucosylated anthocyanidins derived from cyanidin (Pale, 1998). This study was prompted by the need to establish a scientific basis for the ethnomedicine use and preparation of pharmacognostic profile on the plant.

## MATERIALS AND METHODS

### Plant collection

The leaves of *I. asarifolia* were collected from Idu Industrial forest Abuja in May 2003. The plant was authenticated at the Ethnobotany and Herbarium section of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. The voucher specimen (NIPRD) 5396 was deposited at the Herbarium.

### Preparation of plant extract

The extract was obtained by Soxhlet extraction using 84.6 g of the powdered leaves in 500 ml of 70% methanol. The resulting extract

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was concentrated using rotary evaporator and dried on a water bath resulting in a 21% yield.

### Animals

Male and female Swiss albino mice (28.1 - 36.5 g) and Wistar rats (114.0 - 219.0 g) were bred and maintained in the Animal Facility Centre (AFC), Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. They were maintained under normal environmental conditions ( $\pm 37^{\circ}\text{C}$ ) and 12 h day and night illumination cycle. They were fed *ad libitum* with NIPRD formulated standard feed and water from the Municipal Area Council.

### Chemicals

Aspirin (Sigma, USA), formaldehyde 40% w/v (M & B, England), Glacial acetic acid (Searle, Essex, England) and Triton X-100 (Lubley, West Yorkshire, England) were used for the animal studies.

### Phytochemical screening

The presence of various phytochemical constituents in the extract was determined using the standard screening tests outlined in AOU/STRC (1986).

### Acute toxicity studies

The modified method of Lorkes (1983) was employed for the determination of the median lethal dose ( $\text{LD}_{50}$ ). Swiss albino mice were starved of feed but allowed access to water 24 h before the study. They were treated intraperitoneally with different doses of the extract (100, 250, 500, 1000, 1500 and 2000 mg/kg) and were observed for behavioral changes such as ataxia, dullness, excitement, nervousness or death. The  $\text{LD}_{50}$  was then calculated as the geometric mean of the dose that caused 100% mortality and the dose that caused 0% mortality.

### Acetic acid-induced writhing in mice

The test was conducted using the method described by Koster et al. (1959). The mice were divided into five groups ( $n = 5$ ). Mice in group I were pre-treated with normal saline (10 ml/kg i.p.). Mice in groups II, III and IV received the extract (100, 200 and 400 mg/kg i.p.) respectively while mice in group V received aspirin (100 mg/kg i.p.). 0.75 % glacial acetic acid (10 ml/kg i.p.) was then administered to each mouse 30, 60, 90 and 120 min after the pre-treatment. Each mouse was placed in transparent observation box. Five minutes after the acetic acid administration, the number of writhes (contortions of the abdominal muscles) that occurred within 10 min was counted using a counter. The percentage writhes for the treated group were calculated in relation to the control group. The activity was also expressed as percent inhibition of nociception (reduction in episodes of writhing between saline control and treated groups).

### Formalin test

The procedure of Dubuisson and Dennis (1977) was adopted for this study. Four groups of rats (of five rats each) were pre-treated respectively with the extract (100, 200, 400 mg/kg i.p.) and normal saline (10 ml/kg i.p.). 30 min post-treatment, 50  $\mu\text{l}$  (0.05 ml) of 2.5%

formalin was injected into the sub-plantar surface of the left hind paw of each rat. The severity of pain was rated and scored as follows:

- 0 = rat stood firmly or walked on the paw
- 1 = partially elevated or favoured the paw
- 2 = elevated the paw off the floor
- 3 = licked, bit or shook the paw

The cut off point for the observations was every 2 min for the first 10 min post-formalin administration (for early nociceptive phase) and at every 5 min between the next 10 to 60 min intervals (for late nociceptive phase).

### Anti-inflammatory study

Fresh egg albumin induced oedema model of Akah and Nwambie (1994) was employed. The Wistar rats used for the investigation were fasted overnight. They were deprived of water during the experiment to ensure uniform hydration and minimize variability in oedematous response (Winter et al., 1963). Extract (100, 200, 400 mg/kg i.p.), aspirin (100 mg/kg i.p.) and normal saline (10 ml/kg i.p.) were administered to different groups of the rats ( $n = 5$ ). Inflammation was then induced 30 min later by injecting 0.1 ml of fresh egg albumin into the sub-plantar surface of the right hind paw of each of the rats. The principle of volume displacement was adopted for the measurement of paw volume ( $\text{cm}^3$ ) using LETICA Digital plethysmometer (LE 7500) earlier calibrated with 0.15% Triton X-100. Readings were taken before the injection of egg albumin and 20 min intervals after the injection of egg albumin over a 2 h (120 min) period. The oedema at every interval was calculated in relation to the paw volume before the injection of the egg albumin. Activity for the treated groups was expressed as percent inhibition of inflammation in relation to the control group.

### Statistical analysis

The results were expressed as mean  $\pm$  SEM. Statistical significance between groups was analyzed using two – way analysis of variance (ANOVA). Duncan's test (multiple range test) was employed for further analysis. P values ( $\leq 0.05$ ) were considered to be statistically significant.

## RESULTS

### Phytochemical Screening

Methanol leaf extract of *I. asarifolia* contains saponin, tannin, alkaloid and phenol. Cardiac and glycosides, flavonoid, volatile oil and terpenes were absent (Table 1).

### Acute toxicity study ( $\text{LD}_{50}$ )

No behavioral changes were observed in the mice at doses  $\leq 1000$  mg/kg i.p. At 1500 mg/kg i.p. dose, there was dullness and shivering but no mortality occurred 24 h post-treatment. However, at 2000 mg/kg i.p. dose, there was dullness, shivering and total mortality within 24 h of treatment. The derived  $\text{LD}_{50}$  was 1732.1 mg/kg i.p. in mice.

**Table 1.** Result of phytochemical screening of *Ipomea asarifolia* leaf.

Test	Observation	Inference
Saponin	A slight frothing occurred	++
Cardiac glycoside	Green precipitate was observed	--
Digitalis glycosides	A light brown layer was formed	--
Tannins	A light brown brown precipitate was observed	++
Flavonoids	A dirty green colouration was observed	--
Alkaloids Wagner's Reagent	A deep brown precipitate was formed	++
Alkaloid Dragendoff's Reagent	Deep brown precipitate was formed	++
Volatile oil	A green coloured solution was observed	--
Phenols	A bluish green colouration was observed	++
Terpenes	Brown colouration of the top solution was observed.	--
Sterols	A reddish brown interphase was observed with a light green top layer and a light brown lower layer.	+

– : absent; + : slightly present; ++ : moderately present

**Table 2.** Effect of methanolic extract of *Ipomea asarifolia* leaf on acetic acid-induced writhing in mice.

Treatment (mg/kg i.p.)	Number of writhes			
	30 min	60 min	90 min	120 min
Normal saline (10 ml/kg i.p.)	85.2 ± 1.7	66.6 ± 3.1	70.2 ± 1.7	62.0 ± 5.5
<i>I. asarifolia</i>				
100	27.8 ± 7.1*	15.6 ± 1.8*	8.8 ± 3.8*	22.8 ± 1.5*
200	6.8 ± 1.6*	13.6 ± 1.9*	21.6 ± 4.5*	4.2 ± 1.4*
400	19.2 ± 1.9*	15.2 ± 2.2*	5.6 ± 1.5*	2.8 ± 0.4*
Aspirin (100 mg/kg i.p.)	11.0 ± 1.0*	17.0 ± 0.6*	44.4 ± 3.8*	39.8 ± 2.3*

\*Significant (P < 0.05) reduction of writhes; 2-way ANOVA and Duncan (multiple range test).

### Acetic acid-induced writhing in mice

The leaf extract of *I. asarifolia* (100, 200, 400 mg/kg i.p.) showed a significant (P < 0.05) antinociceptive effect in the treated mice (Table 2). The anti-nociceptive effect was dose dependent having a dose-pain reduction effect of 73.6, 83.7 and 84.9% for 100, 200 and 400 mg/kg i.p. of the extract respectively over 120 min. The maximal antinociceptive effect for 100 mg/kg dose was at 90 min having writhing of 12.5% (equivalence of pain inhibition of 87.5%) while those of 200 and 400 mg/kg dose were both at 120 min with writhes of 6.8 and 4.5% (equivalence of pain inhibition of 93.2 and 95.5%) respectively. The result was comparable to that of aspirin (100 mg/kg i.p., Table 3).

### Formalin -induced pain

The extract reduced the severity of formalin-induced pain only in the late phase (15 – 60 min). This effect was signi-

ficant (P<0.05) at 200 mg/kg i.p dose (Table 4).

### Anti-inflammatory properties of *I. asarifolia*

Extract of *I. asarifolia* caused significant (P < 0.05) reduction of the egg-albumin induced inflammatory process at variable doses (100 – 400 mg/kg i.p.). Oedema inhibition 33.3 and 37.5% was exerted by extract doses of 100 and 400 mg/kg i.p respectively. This inhibitory effect was only observed 20 min post-treatment for the dose of 200 mg/kg i.p. The observed inhibitory effects were also variably significant (P < 0.05) over the 120 min observation time (Figure 1).

### DISCUSSION

The intraperitoneal LD<sub>50</sub> of the extract 1732.1 mg/kg may indicate that it is relatively safe. Lorkes (1983) considered LD<sub>50</sub> value greater than > 1 g as safe. However, the behavioral changes (shivering, dullness) that occur-

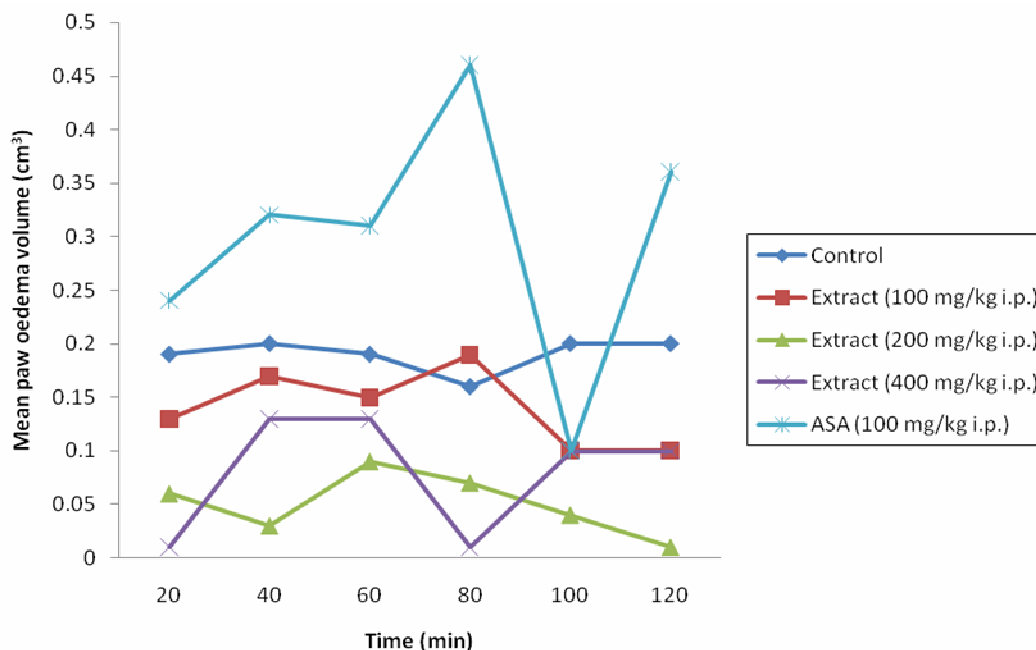
**Table 3.** Effect of methanolic extract of *Ipomoea asarifolia* leaf on early and late phases of formalin-induced pain in rats.

Treatment (mg/kg i.p.)	Early phase(0 – 10 min)		Late phase(15 – 60 min)	
	Score of pain	% Pain inhibition	Score of pain	% Pain inhibition
Normal saline (10 ml/kg i.p.)	8.4 ± 2.1	–	23.6 ± 1.5	–
<i>I. asarifolia</i>				
100	16.0 ± 0.5	–	18.2 ± 1.2	22.9
200	11.2 ± 1.3	–	15.2 ± 1.2	35.6
400	11.2 ± 0.7	–	21.6 ± 1.1	8.5

**Table 4.** Anti-inflammatory study of *Ipomoea asarifolia*.

Treatment	Time (min)					
	20	40	60	80	100	120
Control	0.19 ± 0.36	0.2 ± 0.45	0.19 ± 0.48	0.16 ± 0.46	0.2 ± 0.57	0.2 ± 0.56
Extract (100 mg/kg i.p.)	0.13 ± 0.26*	0.17 ± 0.14*	0.15 ± 0.27*	0.19 ± 0.54	0.1 ± 0.32*	0.1 ± 0.38*
(200 mg/kg i.p.)	0.06 ± 0.28*	0.03 ± 0.40*	0.09 ± 0.49	0.07 ± 0.54	0.04 ± 0.64	0.01 ± 0.58
(400 mg/kg i.p.)	0.01 ± 0.13*	0.13 ± 0.23*	0.13 ± 0.37*	0.01 ± 0.45	0.1 ± 0.31*	0.1 ± 0.58*
ASA (100 mg/kg i.p.)	0.24 ± 0.02*	0.32 ± 0.1*	0.31 ± 0.1*	0.46 ± 0.1	0.1 ± 0.34*	± 0.1*

\* P < 0.05 when compared to normal saline treated group.

**Figure 1.** Effect of leaf extract of *Ipomea asarifolia* on egg album-induced oedema in rats.

red at doses 1500 mg/kg i.p. possibly indicated onset of toxicity, hence, the need for a careful dose selection. A sub-chronic data may be sufficient to predict the hazard on a long term, which is low dose exposure to a particular compound (McNamara, 1976). The anti-nociceptive effect of the aqueous methanolic extract of *I. asarifolia* leaf was

shown by its ability to reduce the number of acetic acid-induced abdominal constrictions (writhes) in mice. Collier et al. (1968) and Bentley et al. (1981) have shown this test to be very sensitive and able to detect anti-nociceptive effects of compounds as dose levels that may be inactive in other methods like the tail – flick test. Local pe-

ritoneal receptors may be partly involved in the abdominal constriction response (Bentley et al., 1983) caused by peritoneal fluid concentration of PGE<sub>2</sub> and PGF<sub>2α</sub> (Deraedt et al., 1980). Although the mechanism of action of the extract is not yet clear, it is possible that it reduced the receptor sensitivity to the chemical (acetic acid-induced pain) in a dose dependent manner. The high percent pain inhibition, 93.2 and 95.5%, exhibited by the extract at 200 and 400 mg/kg i.p. doses respectively up to 120 min. shows prolonged anti-nociceptive property.

Reduction in the formalin-induced pain by the extract took place in the late phase (15 - 60 min) of the process. According to Dubussion and Dennis (1977) and Tjolsen et al. (1992), nociception occur in two phases in formalin test. The first phase occurs immediately after formalin injection and continues for 5 min, after which nociception appears to diminish. In the second phase, a high level of nociception begins 15 – 20 min after formalin injection and continues for approximately 60 min. The first phase is probably a direct result of stimulation of paw nociceptors, while the second phase may reflect the inflammation process, and at least to some degree, the sensitization of central nociceptive neurons (Coderre et al., 1990; Coderre and Melzack, 1992). This method has been found useful for clarifying the mechanism of pain and analgesia (Tjolsen et al., 1992). Thus the extract may have either interfered with the inflammatory process or inhibited sensitization of central nociceptive neuron or both since its activity was in the late phase. Drugs such as aspirin, hydrocortisone and dexamethasone which primarily exert peripheral action similarly inhibit only the late phase (Chen et al., 1995; Elisabetsky et al., 1995; Santos et al., 1995). This study which used the *in vivo* model for acute inflammation (Akah et al., 1993; Akah and Nwambie, 1994) showed that the extract reduced egg-albumin induced oedema induced in rats. This effect was however not dose-related. Inflammatory process is caused by the release of mediators from tissues and migrating cells by the prostaglandins (PG<sub>s</sub>), leucotrienes (LT<sub>s</sub>), histamine, bradykinin, platelet-activating factor (PAF) and interleukin – 1 (Vane and Botting, 1990). This suggests that the extract possibly inhibited the mediators of inflammation leading to reduction in oedema which further corroborates its activity in the late phase of formalin test. Overall, the extract has both anti-nociceptive and anti-inflammatory properties. Both effects are useful in therapeutics for various non-steroidal anti-inflammatory drugs (NSAIDs) especially salicylates and their congenes.

The leaf extract contains different classes of compounds, such as carbohydrates, tannins, saponins, terpenes and steroids. Tannins are organic substances of diverse composition with pronounced astringent properties that hasten the healing of wounds and inflamed mucous membranes. Saponin also exhibits expectorant action which is useful in reducing inflammation of the upper respiratory passages (Starry and Storchova, 1998). These phytochemical constituents may contribute to the anti-

nociceptive and anti-inflammatory activities of *I. asarifolia*.

## Conclusion

This study supports the traditional use of *I. asarifolia* leaves in pain relief, anti inflammation and dysmenorrheal. It will also contribute to the development of monograph on the plant towards standardization and drug development.

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