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ISOLATION, ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF *SALIX LUCIDA* MUHL (SALICACEAE) LEAF METHANOL EXTRACT IN SWISS ALBINO MICE

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ABSTRACT

The use of plants for medicinal purposes has revolutionized in recent times. Many plants have been used to treat sicknesses like cancers, inflammations, pains, piles, high blood pressure, malaria fever, viral infections, and bacterial infections. One of such plant is *Salix lucida* (shinning willow). It has been used in treating pains and inflammations in traditional medicine in Nigeria. The aim of this present study was to evaluate the anti-inflammatory and analgesic potentials of *S. lucida* leaf extract, and isolate the bioactive compound that might be responsible for these activities. *Salix lucida* leaf methanol extract was screened to determine anti-inflammatory and analgesic activities in Swiss albino mice in carrageenan induced paw edema and acetic acid induced pain respectively at different extract doses of 400, 800, and 1,200mg/kg b.w. GC-MS and NMR spectroscopy were used to characterized the bioactive compound. The results showed that at dosage of 1,200mg/kg b.w., there were significant reductions in inflammation and analgesia in the mice treated with *S. lucida* leaf extract, with pronounced reductions in the volume of paw edema when compared to the mice treated with standard drugs aspirin and ibuprofen respectively for 6 hours ($p<0.05$; one-way ANOVA). Mass spectra and NMR analysis revealed the presence of a flavonoid compound as the bioactive material which might be responsible for the observed biological activities. Therefore, the findings from this study justified the use of the leaf extract of *Salix lucida* in the treatment of inflammation and pains (analgesia) in the Nigerian traditional medicine.

KEYWORDS

Salix lucida, Isolation, Anti-inflammatory, Analgesic and Paw-edema.

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INTRODUCTION

It has been stated that inflammation means the act of responding to injury by cells and tissues by many mechanisms while analgesia is a form of stimuli by the body which resulted from damaged cells or tissues[1]. Because, the treatment of inflammations and pains are effected through the use of specific

inhibitors of some metabolic pathways like the cyclo-oxygenase which involved the production of prostaglandins, modern non-steroidal anti-inflammatory drugs (NSAIDs) and analgesics are used[2]. Prolonged usage of these drugs to manage swellings and pains have resulted in many form of resistances and some side effects in patients[3-6].

Hence, in attempt to seek for an alternative therapeutic measure for inflammations and pains, researchers have found solace in medicinal or herbal plants. It is believed that these plants are essential sources of new bioactive substances against inflammations and pains with low or no toxicological effects[7]. In Nigeria, especially, in Sarduana Local Government Area of Taraba State, various plants have been used to treat inflammation and pain by traditional medicine practitioners, many of which the claims have not been verified scientifically. One of such medicinal plant popularly used for the purpose of reducing inflammations and pains (analgesia) is *Salix lucida* (the shinning willow).

Salix lucida is a perennial shrub or tree. It is known by various names such as the shining willow, pacific willow, and the whiplash willow. It belongs to the species of willow which is native to the Americans (North and North-west). The plant is also distributed in countries with high amount of rainfall and found along river and stream banks and wetlands of Nigeria, Ghana, Cote devoir, Cameroun, and Zimbabwe[8-11]. It is an evergreen shrub or medium tree whose height rages between 3-10m high. The narrow leaves are glossy and shining, taking the form of *Amaranthus viridis*. Its stem is greenish, flowers are yellow, and hairless[12]. The leaf has been reported to be used as astringent, analgesic, anti-asthma, anti-bleeding, antipyretic, stomach and tooth-ache remedies[12-15]. Like most willows, the bark is bitter in taste, and contain an aspirin-like compound for which reason willows are powerful anti-inflammatory agents[16].

Although, most willows contain flavonoids, catechins and phenolic, the phytochemical composition of *S. lucida* has not been determined to the best of our knowledge. Therefore, the use of gas chromatography-mass spectrometry (GC-MS) as well as nuclear magnetic resonance (NMR) spectroscopy will reveal to some extent the phytoconstituents of *S. lucida* leaf extract. This present study was carried out to determine the anti-inflammatory and analgesic activities of *S. lucida* in mice with a view to justify its acclaimed used in the treatment of inflammations and pains in traditional medicine, and determine the bioactive compound of leaf extract for the observed bioactivities in the mice.

MATERIAL AND METHODS

Collection of and preparation of plant material

Fresh leaves of *Salix lucida* were collected in the morning hours from a wetland area in Nguroje, Taraba State, Nigeria in August 2018, and was authenticated by a taxonomist. A voucher specimen number *SAL001* was deposited for the plant at the medicinal plant herbarium. The leaves were then air-dried under shade for two weeks, and reduced into fine powder using electronic blender (made in China). The powder was then put in an air-tight polythene bag and kept in a refrigerator at 45^{o}C for further use.

Preparation of plant extract

Exactly 800g of powdered *S. lucida* leaves were extracted by cold maceration technique in 2.5 Litres of 98.2% (v/v) methanol (Sigma Aldrich, USA) for 72 h. The filtrate was then concentrated in vacuole in a rotary evaporator (England) to obtain a dark gel-like extract or residue. The extract was weighed and the percentage yield of 16% was obtained, and then stored in a clean dry sample bottle, and kept in refrigerator at -4^{o}C for further use.

Animals and experimental design for anti-inflammatory and analgesic studies

Sixty male Swiss albino mice weighing between 15-25g were purchased from the animal house of the Department of Pharmacology, University of Jos, Nigeria. The animals were kept in plastic cages with free access to water and food and in standard weather conditions as approved by the University's research ethic committee and international guidelines for animals in research.

Experimental design for anti-inflammatory study

Thirty Swiss albino mice were randomly grouped into five groups of six mice per group as shown below:

Group 1, was the negative control group which received 0.5mL normal saline (i.p.).

Group 2, was the positive control group which received 300mg/kg aspirin (i.p.).

Group 3, received 400mg/kg b.w. *Salix lucida* leaf extract (SLLE). (i.p.).

Group 4, received 800mg/kg b.w. SLLE (i.p.).

Group 5, received 1,200mg/kg b.w. SLLE (i.p.).

Experimental design for analgesic study

Thirty Swiss albino mice were randomly grouped into five groups of six mice per group as shown below:

Group 1, was the negative control group which received 0.5mL normal saline (i.p.).

Group 2, was the positive control group which received 200mg/kg ibuprofen USP (i.p.).

Group 3, received 200mg/kg b.w. *Salix lucida* leaf extract (SLLE). (i.p.).

Group 4, received 400mg/kg b.w. SLLE (i.p.).

Group 5, received 600mg/kg b.w. SLLE (i.p.).

Chemicals and solvents used

Methanol, n-hexane, ethyl acetate, acetic acid, and carrageenan, were purchased from Joe Chem Nigeria Ltd a distributor of Sigma Chemical Co., (St. Louis Mo, USA), aspirin and ibuprofen were purchased from Jude Pharmacy, Bali, Nigeria. The remaining chemicals and solvents were of analytical grades.

Anti-inflammatory activity study

Carrageenan-induced paw edema in mice model

Paw edema was induced in the mice injection of 0.1mL carrageenan (in 1% normal saline) following intradermal route into the sub-plantar area of the right hind paw of the mice[17]. The paw edema volume was then measured using a plethysmometer before 0, 1, 2, 3, and hours after injecting the mice with carrageenan. The plant extract and the control drug were given to the mice (i.p.) an hour before carrageenan was used to induce paw edema. The percentage inhibition of paw edema was then calculated from the formula below:

% inhibition of edema = $[(C_o-C_t)/C_o] \times 100$ (i)

Where, C_o is the mean value of inflammation (in hind paw edema) of the control group at a given hour, and C_t is the mean value of inflammation of the plant extract[18-20].

Analgesic study of extract

Acetic acid induced writhing in mice study

In this study, standard drug and plant extract doses were administered (i.p.) to mice and 0.1mL of 1% acetic acid (v/v) solution was administered 30 min later (i.p.). The animals were each placed in separate beakers and then observed for 10 min for abdominal writhing by turning the mice upside down and viewing them using a magnifying glass[21-22]. The percentage of inhibition of abdominal writhing in mice was calculated from the formula below:

% inhibition = (average no of writhing $_{control}$ - average no of writhing $_{treated}$ /average no of writhing $_{control}$) 100 …….. (ii)

Analgesic activity study by tail flick response in mice

The albino mice were placed separately on an analgesiometer while the tail were freely emerging from the holder. The middle part of tail of each mice was placed along the heat source of the apparatus. A quick removal of tail by the mice from the source of heat which is the tail-flick response was regarded as the end of the investigation. The reaction time was the time when the tail was placed on the heat source and the time the mice quickly removed its tail from the heat source[22]. Each mouse was tested three times while, allowing 5 min gap between each responses and means of all values were taken as latent periods. Tested animals were marked and kept in separate cages. Tail-flick test was carried out in 30, 60, 90, and 120 min after extract administration and before. The percentage of inhibition of analgesia (pain) was calculated from the formula below:

% inhibition of analgesia = (Tla–Bla/Mla–Bla) 100 (iii)

Where, Tla = test latency at the end of testing for each mouse, Bla = basal latency, Mla = maximum latency (10s).

Phytochemical screening of *S. lucida* leaf extracts

The phytochemical constituents of the leaf extract were determined following the methods previously described by Trease and Evans as well as Sofowora[23,24] for the presence of some secondary metabolites in various fractions of leaf extract.

NMR and GC-MS analysis of extract

NMR analysis of bioactive compound was recorded on a Bruker NMR 600MHZ with deuterated chloroform ($CDCl_3$) as standard solvent for ^{1}H-NMR, COSY-NMR, ^{13}C-NMR, DEPT-135, HSQC and HMBC data while, the GC-MS analysis of compound was carried out on an Agilent Technologies 7890A GC coupled to a MS detector with DB23 column model number J and W 1222362, and internal diameter of 60m × 250µm × 0.25µm (250°C Max).

Statistical analysis

The data obtained from the study was analyzed by using Graph Pad Prism software version 9.1 (2020). Statistical significance difference between groups were expressed using one-way ANOVA. The value of $p < 0.05$ was taken as statistically significant.

RESULTS AND DISCUSSION

Anti-inflammatory and analgesic activities

The results in Figure No.2-4 showed anti-inflammatory and analgesic activities were dose-dependent. For instance, ethyl acetate extract (EF) showed significant decrease in paw edema thickness in the animals from 20 to 10mm within 24 h as doses of SLLE are increase from 400 to 1,200mg/kg b.w. indicating a higher anti-inflammatory potential. This was not case of hexane fraction with increased paw thickness of 30 to 60mm within the same period (Figure No.2). These values are significantly different from that of the control ($p<0.05$; one-way ANOVA). Similar result was obtained for analgesic activity where the EF produced the least number of writhing of abdominal muscle within 4 h while the HF produced the highest (Figure No.3). The percentage inhibition of writhing induced by acetic acid was highest with the EF than any other treatment groups (Table No.1, Figure No.4).

In Figure No.5, the EF and PC showed the least latency time change in tail-flick response in the mice while the HF produced the highest latency time change. These values were significantly different from the control drug ($p<0.05$; one-way ANOVA).

Phytochemicals, NMR and GC-MS analysis of bioactive compound

The preliminary phytochemical screening of the leaf extracts revealed the presence flavonoids, phenolic, alkaloids and terpenoid with no trace of saponins and anthracenes. Isolated bioactive compound from the ethyl acetate fraction showed greenish crystals with the following: melting point; 52.13°C, R_f value; 0.73, 1H-NMR(600 MHZ, $CDCl_3$) data showed eight signals at 1H δ (ppm) 0.8 (t, J= 3.0 Hz), 2H δ 1.0 (q, J= 12.0 Hz), 3H δ 1.07 (s, J= 1.0 Hz), 4H δ 1.13 (s, J = 1.0 Hz), 5H δ 1.34 (q, J = 6.0 Hz), 6H δ 1.49 (q, J = 1.0 Hz), 7H δ 1.74 (q, J = 1.0 Hz), 8H δ 1.8 (s, J= 1.0 Hz); 13C-NMR showed 31 carbon atoms with chemical shift values from 23.0 to 34.0 ppm which are characteristic positions of methine, methyl and methylene carbons. These protons correlated with the carbon atoms as confirmed with COSY, 135-DEPT, HSQC, and HMBC; mass spectra data showed molecular ion of 149mol/g and a base peak of 120 which corresponds with 2-ethyl formanilide.

Discussion

Nowadays, herbal plants have continued to be used as an interesting remedy for various diseases in the health-care system. An estimated 150,000 species of medicinal plants from various families have been researched and were found to possessed compounds with biological properties against certain diseases. Some of these compounds have been used as active ingredient if some pharmaceutical formulations that are currently used in todays' health-care delivery system. Medicinal plants have continued to play significant role in Nigerian traditional medicine for decades as anti-cancer, anti-pyretic, anti-

inflammatory, anti-ulcer, analgesic, anti-diabetic, anti-diarrhoea, anti-hypertensive agents, among others[25]. The use of morphological and other parts of plant is the most common type of traditional medicine in Nigeria.

In this current study, *Salix lucida* (Figure No.1) leaf methanol extract was evaluated for its anti-inflammatory and analgesic activities in albino mice. The study showed that the *S. lucida* leaf extract (SLLE) decreased carrageenan induced-paw edema in mice within the period of study. Ethyl acetate fraction (EF) produced the least volume of paw edema as compared to that produced by n-hexane fraction (HF) in the mice (Figure No.2). These decreases were in dose-dependent fashion because, at the dose of 1200 mg/kg there was significant reduction in paw volume in the mice. These results were comparable to that of the control drug aspirin ($p<0.05$; one-way ANOVA).

It can be said authoritatively that the inhibition of paw edema caused by carrageenan may be due to the presence of certain secondary metabolites such as flavonoids, phenolic and terpenes. For instance, flavonoids have been reported to possess arrays of biological properties such as anti-oxidant, anti-inflammatory, anti-cancer and anti-pyretic[26]. *Salix lucida* leaf extracts must have achieve this therapeutic action in the mice by suppressing the activities of cyclooxygenase enzyme from making prostaglandins which is responsible inflammation. Similarly, the reduction in abdominal writhing caused by acetic acid in the mice was dose-dependent (Figure No.3, 4, Table No.1). These highest reductions were witnessed in the ethyl acetate fraction (EF) and the pure compound (PC) as compared to the nociceptive effects produced by the standard drug ibuprofen tablet.

It is also possible that the extracts achieved these therapeutic potentials by mediating using the peripheral and the central nervous system by inhibiting the nerve impulses that will lead to the synthesis of prostaglandins (PGS). In all, *S. lucida* leaf extract was able to show its ability to reduce pains in the mice by lowering the changes in latency time in the tail-flick test for over a period of 6 hours. The ethyl acetate fraction (EF) and the isolated pure compound (PC) showed the least changes in latent time of all the extracts (Figure No.5). The effectiveness of EF was obviously due to the presence of major metabolites in the extract with key therapeutic potential. This is similar to the report obtained by other researchers when they investigated other species of *Salix* as well as other plant extracts for *in vivo* anti-inflammatory and analgesic activities[27]. For instance, the study revealed the presence of 2-ethyl formanilide (a formanilide-flavonoid derivative) in the ethyl acetate fraction. Techniques such as NMR (1D and 2D), and GC-MS have been used to characterized and elucidate the structure of organic compounds. This is the case in this present study. It has been reported that this isolated compound has been used as an anaesthetic, anti-allergic, antibiotic, antihistamines and antimicrobial agents[28]. Therefore, the analgesic and anti-inflammatory activities displayed by *S. lucida* extracts was due to the presence of this compound.

The study therefore, showed that *Salix lucida* leaf extract possessed both anti-inflammatory and analgesic activities in mice in dose-dependent fashion. However, these activities were mostly exhibited with the isolated pure compound and the ethyl acetate fraction. The study further justified the use of *S. lucida* leaf extract to treat inflammation and pains in traditional medicine in Nigeria.

Table No.1: Effect of methanol leaf extracts of *Salix lucida* on acetic acid-induced abdominal writhing in mice; n= 5

S.No	Group dose (mg/kg)	Number of writhing (mean ± SE)	Inhibition (%)
1	N/saline (mL)	28.03 ± 0.52	-
2	Std. drug	6.89 ± 0.01	75.42
3	N-hexane extract (HF)	22.05 ± 0.20*	21.33
4	Ethyl acetate extract(EF)	11.01 ± 0.42*	60.72
5	Aqueous extract (AF)	18.13 ± 0.21*	35.32
6	Pure compound (PC)	8.01 ± 0.01*	71.42

Results are recorded as mean ± SE; *p<0.05; one-way ANOVA followed by Duncan's multiple range test. All experiments were in triplicates

Figure No.1: Pictorial view of *Salix lucida* in its natural habitat

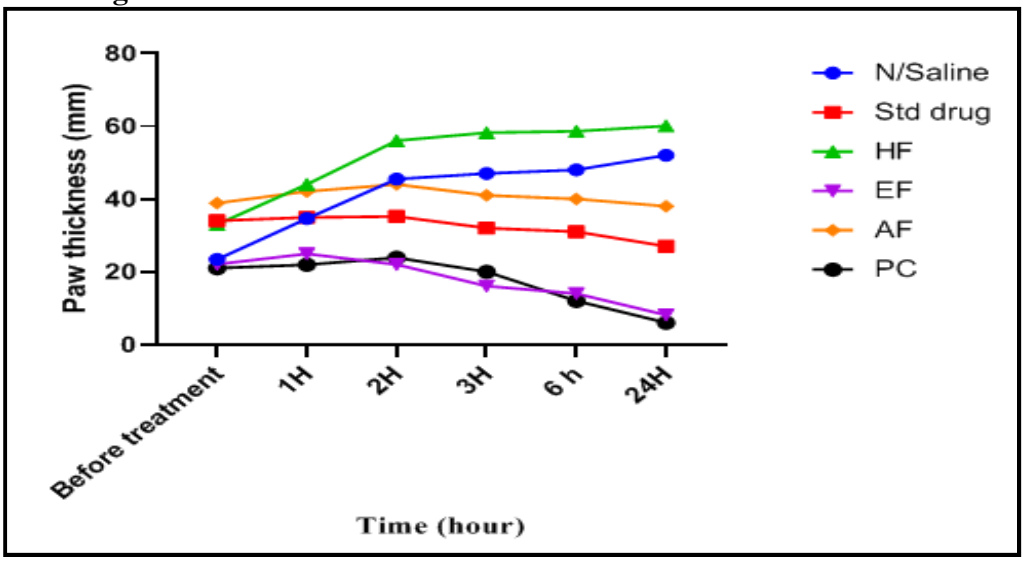

Figure No.2: Paw edema thickness of rat groups within 4 h

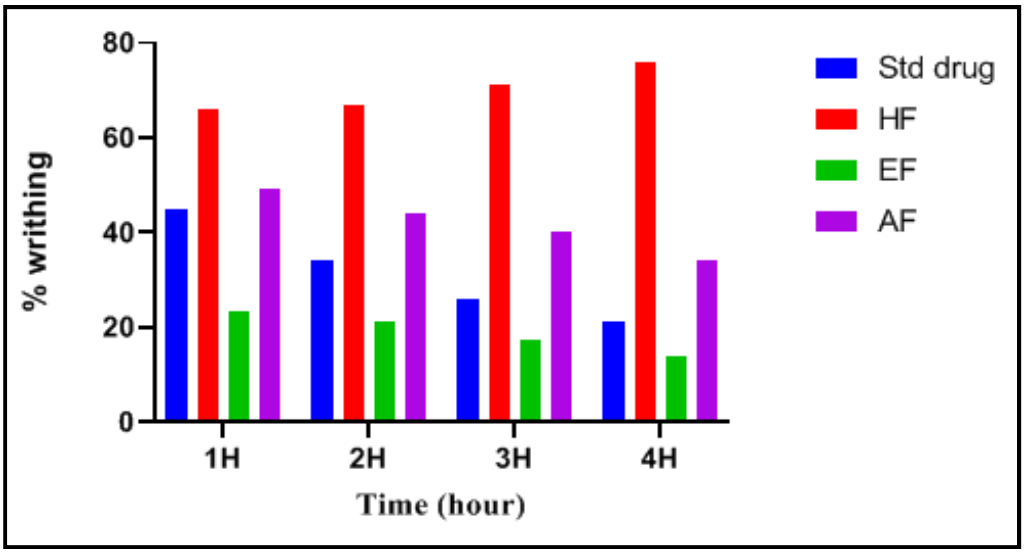

Figure No.3: Effects of *Salix lucida* extracts on abdominal writhing in mice groups

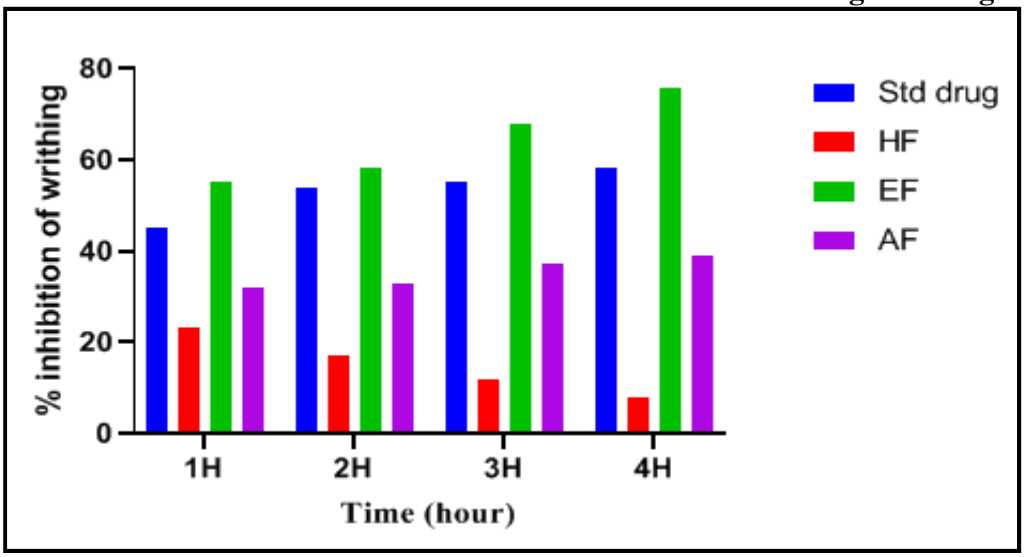

Figure No.4: Effect of *S. lucida* extracts on inhibition (%) of abdominal writhing in mice

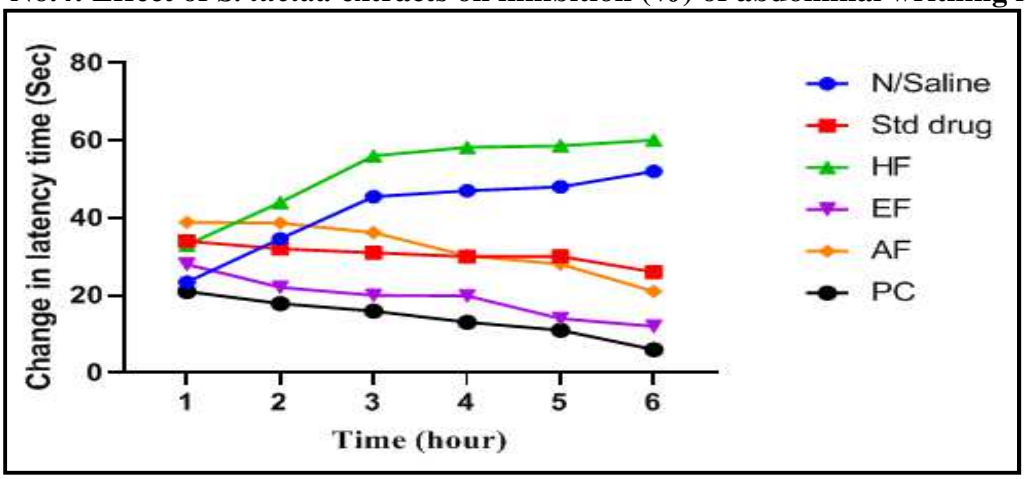

Figure No.5: Effect of various fractions on latency time change in tail-flick response in mice

CONCLUSION

The present study showed that *Salix lucida* leaf extract possessed strong anti-inflammatory and analgesic activities in mice in dose-dependent manner. The study further revealed the presence of a formanilide derivative of flavonoid from the ethyl acetate fraction of the extract as the bioactive compound. However, the mechanism of anti-inflammation and analgesic actions of this compound need to be further studied. Hence, *Salix lucida* leaf extract can be used as an ethno-medicinal prescription for inflammation and pains in traditional medicine. This study therefore, justified its acclaimed used in the treatment of these conditions in traditional medicine in Nigeria.

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COMPETING INTEREST

We have none to declare.

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